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<p>(21) International Application Number: PCT/IB95/00156</p> <p>(22) International Filing Date: 23 February 1995 (23.02.95)</p>		<p>(72) Inventors: STINCHCOMB, Dan, T.; 7203 Old Post Road, Boulder, CO 80301 (US). CHOWRIRA, Bharat; 3250 O'Neal Circle, B-25, Boulder, CO 80301 (US). DIRENZO, Anthony; 1197 Ravenwood Road, Boulder, CO 80303 (US). DRAPER, Kenneth, G.; 4619 Cloud Ct., Boulder, CO 80301 (US). DUDYCYZ, Lech, W.; 24 A Gates Road, Worcester, MA 01603 (US). GRIMM, Susan; 6968 1/2 S. Boulder Road, Boulder, CO 80303 (US). KARPEISKY, Alexander; 5121 Williams Fork Trail #209, Boulder, CO 80301 (US). KISICH, Kevin; 2451 Jonquil Circle, Lafayette, CO 80026 (US). MATULIC-ADAMIC, Jasenka; 760 South 42nd Street, Boulder, CO 80303 (US). McSWIGGEN, James, A.; 4866 Franklin Drive, Boulder, CO 80301 (US). MODAK, Anil; 3855 Hauptman Court, Boulder, CO 80301 (US). PAVCO, Pamela; 705 Barberry Circle, Lafayette, CO 80026 (US). BEIGELMAN, Leonid; 5530 Colt Drive, Longmont, CO 80503 (US). SULLIVAN, Sean, M.; 850 Marina Village Parkway, Alameda, CA 94501 (US). SWEEDLER, David; 956 St. Andrews Lane, Louisville, CO 80027 (US). THOMPSON, James, D.; 2925 Glenwood Drive #301, Boulder, CO 80301 (US). TRACZ, Danuta; 6200 Habitat #3029, Boulder, CO 80301 (US). USMAN, Nassim; 2954 Kalmia #37, Boulder, CO 80304 (US). WINCOTT, Francine, E.; 7920 N. 95th Street, Longmont, CO 80501 (US). WOOLF, Tod; 18 Fairview Avenue, Watertown, MA 02172 (US).</p>																																																																														
<p>(30) Priority Data:</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 30%;">08/201,109</td> <td style="width: 40%;">23 February 1994 (23.02.94)</td> <td style="width: 30%;">US</td> </tr> <tr> <td>08/218,934</td> <td>29 March 1994 (29.03.94)</td> <td>US</td> </tr> <tr> <td>08/222,795</td> <td>4 April 1994 (04.04.94)</td> <td>US</td> </tr> <tr> <td>08/224,483</td> <td>7 April 1994 (07.04.94)</td> <td>US</td> </tr> <tr> <td>08/228,041</td> <td>15 April 1994 (15.04.94)</td> <td>US</td> </tr> <tr> <td>08/227,958</td> <td>15 April 1994 (15.04.94)</td> <td>US</td> </tr> <tr> <td>08/245,736</td> <td>18 May 1994 (18.05.94)</td> <td>US</td> </tr> <tr> <td>08/271,280</td> <td>6 July 1994 (06.07.94)</td> <td>US</td> </tr> <tr> <td>08/291,932</td> <td>15 August 1994 (15.08.94)</td> <td>US</td> </tr> <tr> <td>08/291,433</td> <td>16 August 1994 (16.08.94)</td> <td>US</td> </tr> <tr> <td>08/292,620</td> <td>17 August 1994 (17.08.94)</td> <td>US</td> </tr> <tr> <td>08/293,520</td> <td>19 August 1994 (19.08.94)</td> <td>US</td> </tr> <tr> <td>08/300,000</td> <td>2 September 1994 (02.09.94)</td> <td>US</td> </tr> <tr> <td>08/303,039</td> <td>8 September 1994 (08.09.94)</td> <td>US</td> </tr> <tr> <td>08/311,486</td> <td>23 September 1994 (23.09.94)</td> <td>US</td> </tr> <tr> <td>08/311,749</td> <td>23 September 1994 (23.09.94)</td> <td>US</td> </tr> <tr> <td>08/314,397</td> <td>28 September 1994 (28.09.94)</td> <td>US</td> </tr> <tr> <td>08/316,771</td> <td>3 October 1994 (03.10.94)</td> <td>US</td> </tr> <tr> <td>08/319,492</td> <td>7 October 1994 (07.10.94)</td> <td>US</td> </tr> <tr> <td>08/321,993</td> <td>11 October 1994 (11.10.94)</td> <td>US</td> </tr> <tr> <td>08/334,847</td> <td>4 November 1994 (04.11.94)</td> <td>US</td> </tr> <tr> <td>08/337,608</td> <td>10 November 1994 (10.11.94)</td> <td>US</td> </tr> <tr> <td>08/345,516</td> <td>28 November 1994 (28.11.94)</td> <td>US</td> </tr> <tr> <td>08/357,577</td> <td>16 December 1994 (16.12.94)</td> <td>US</td> </tr> <tr> <td>08/363,233</td> <td>23 December 1994 (23.12.94)</td> <td>US</td> </tr> <tr> <td>08/380,734</td> <td>30 January 1995 (30.01.95)</td> <td>US</td> </tr> </table>		08/201,109	23 February 1994 (23.02.94)	US	08/218,934	29 March 1994 (29.03.94)	US	08/222,795	4 April 1994 (04.04.94)	US	08/224,483	7 April 1994 (07.04.94)	US	08/228,041	15 April 1994 (15.04.94)	US	08/227,958	15 April 1994 (15.04.94)	US	08/245,736	18 May 1994 (18.05.94)	US	08/271,280	6 July 1994 (06.07.94)	US	08/291,932	15 August 1994 (15.08.94)	US	08/291,433	16 August 1994 (16.08.94)	US	08/292,620	17 August 1994 (17.08.94)	US	08/293,520	19 August 1994 (19.08.94)	US	08/300,000	2 September 1994 (02.09.94)	US	08/303,039	8 September 1994 (08.09.94)	US	08/311,486	23 September 1994 (23.09.94)	US	08/311,749	23 September 1994 (23.09.94)	US	08/314,397	28 September 1994 (28.09.94)	US	08/316,771	3 October 1994 (03.10.94)	US	08/319,492	7 October 1994 (07.10.94)	US	08/321,993	11 October 1994 (11.10.94)	US	08/334,847	4 November 1994 (04.11.94)	US	08/337,608	10 November 1994 (10.11.94)	US	08/345,516	28 November 1994 (28.11.94)	US	08/357,577	16 December 1994 (16.12.94)	US	08/363,233	23 December 1994 (23.12.94)	US	08/380,734	30 January 1995 (30.01.95)	US	<p>(74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).</p>
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<p>(71) Applicant: RIBOZYME PHARMACEUTICALS, INC. [US/US]; 2950 Wilderness Place, Boulder, CO 80301 (US).</p>		<p>(81) Designated States: AU, CA, JP, KR, MX, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>Without international search report and to be republished upon receipt of that report.</i></p>																																																																														
<p>(54) Title: METHOD AND REAGENT FOR INHIBITING THE EXPRESSION OF DISEASE RELATED GENES</p> <div style="text-align: center;"> <p>Substrate RNA</p> </div>																																																																																

(57) Abstract

Enzymatic RNA molecules which cleave ICAM-1 mRNA, IL-5 mRNA, *rel A* mRNA, TNF- α mRNA, RSV mRNA or RSV genomic RNA, or CML associated mRNA, and use of these molecules for the treatment of pathological conditions related to those mRNA-levels; ribonucleosides or nucleotides modified in 2', 3' or 5', methods for their synthesis, purification and deprotection; vectors containing multiple enzymatic nucleic acids, optionally in chimeric form with tRNAs; method for introducing enzymatic nucleic acids into cells by forming a complex with a second nucleic acid, where the complex is capable of taking an R-loop base-paired structure; method for altering a mutant nucleic acid *in vivo* by hybridization with an oligonucleotide capable of activating dsRNA deaminase, comprising an enzymatic activity or a chemical mutagen. Further are disclosed trans-cleaving or -ligating hairpin ribozymes lacking a substrate RNA moiety, as well as hammerhead ribozymes having an interconnecting loop between base pairs in stem II.

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METHOD AND REAGENT FOR INHIBITING THE EXPRESSION
OF DISEASE RELATED GENESBackground of the Invention

This invention relates to reagents useful as inhibitors of gene expression relating to diseases such as inflammatory or autoimmune disorders, chronic myelogenous leukemia, or respiratory tract illness.

5

Summary of the Invention

The invention features novel enzymatic RNA molecules, or ribozymes, and methods for their use for inhibiting the expression of disease related genes, e.g., ICAM-1, IL-5, *relA*, TNF- α , p210 *bcr-abl*, and respiratory syncytial virus genes. Such ribozymes can be used in a method for
10 treatment of diseases caused by the expression of these genes in man and other animals, including other primates.

Ribozymes are RNA molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence specific manner. Such enzymatic RNA molecules can be
15 targeted to virtually any RNA transcript, and efficient cleavage has been achieved *in vitro*. Kim et al., 84 Proc. Natl. Acad. Sci. USA 8788, 1987; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acids Research 1371, 1989.

Six basic varieties of naturally-occurring enzymatic RNAs are known
20 presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. Table 1 summarizes some of the characteristics of these ribozymes.

Ribozymes act by first binding to a target RNA. Such binding occurs
25 through the target RNA binding portion of a ribozyme which is held in close proximity to an enzymatic portion of the RNA which acts to cleave the target RNA. Thus, the ribozyme first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a
30 target RNA will destroy its ability to direct synthesis of an encoded protein. After a ribozyme has bound and cleaved its RNA target it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the effective concentration of ribozyme necessary to effect a therapeutic treatment is lower than that of an antisense oligonucleotide. The advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding, but also on the mechanism by which the molecule inhibits the expression of the RNA to which it binds. That is, the inhibition is caused by cleavage of the RNA target and so specificity is defined as the ratio of the rate of cleavage of the targeted RNA over the rate of cleavage of non-targeted RNA. This cleavage mechanism is dependent upon factors additional to those involved in base pairing. Thus, it is thought that the specificity of action of a ribozyme is greater than that of antisense oligonucleotide binding the same RNA site. With their catalytic activity and increased site specificity, ribozymes represent more potent and safe therapeutic molecules than antisense oligonucleotides.

Thus, in a first aspect, this invention relates to ribozymes, or enzymatic RNA molecules, directed to cleave RNA species encoding ICAM-1, IL-5, relA, TNF- α , p210^{bcr-abl}, or RSV proteins. In particular, applicant describes the selection and function of ribozymes capable of cleaving these RNAs and their use to reduce levels of ICAM-1, IL-5, relA, TNF- α , p210 bor-abl or RSV proteins in various tissues to treat the diseases discussed herein. Such ribozymes are also useful for diagnostic uses.

Applicant indicates that these ribozymes are able to inhibit expression of ICAM-1, IL-5, rel A, TNF- α , p210^{bcr-abl}, or RSV genes and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave target ICAM-1, IL-5, rel A, TNF- α , p210^{bcr-abl}, or RSV encoding mRNAs may be readily designed and are within the invention.

These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the

cleavage of RNA. Upon binding, the ribozymes cleave the target encoding mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, a therapeutic effect may be observed.

By "gene" is meant to refer to either the protein coding regions of the cognate mRNA, or any regulatory regions in the RNA which regulate synthesis of the protein or stability of the mRNA; the term also refers to those regions of an mRNA which encode the ORF of a cognate polypeptide product, and the proviral genome.

By "enzymatic RNA molecule" it is meant an RNA molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave RNA in that target. That is, the enzymatic RNA molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. This complementarity functions to allow sufficient hybridization of the enzymatic RNA molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. By "equivalent" RNA to a virus is meant to include those naturally occurring viral encoded RNA molecules associated with viral caused diseases in various animals, including humans, cats, simians, and other primates. These viral or viral-encoded RNAs have similar structures and equivalent genes to each other.

By "complementarity" it is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example, Hoogsteen type) of base-paired interactions.

In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or *Neurospora* VS RNA. Examples of such hammerhead motifs are described by Rossi *et al.*, 1992, *Aids Research and Human Retroviruses*, 8,183, of hairpin motifs by Hampel and Tritz, 1989 *Biochemistry*, 28, 4929, EP 0360257 and Hampel *et al.*, 1990, *Nucleic Acids Res.* 18,299 and an example of the hepatitis delta virus motif is described by Perotta and Been, 1992 *Biochemistry*, 31 16 of the RNaseP motif by Guerrier-Takada *et al.*, 1983 *Cell*, 35 849,

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expressed in eukaryotic cells from the appropriate DNA or RNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO93/23569, and Sullivan et al., PCT WO94/02595, both hereby incorporated in their totality by reference herein; Ohkawa, J., et al., 1992, Nucleic Acids Symp. Ser. 27, 15-6; Taira, K. et al., Nucleic Acids Res., 19, 5125-30; Ventura, M., et al., 1993, Nucleic Acids Res., 21, 3249-55, Chowrira et al., 1994 J. Biol. Chem., 269, 25856).

By "inhibit" is meant that the activity or level of ICAM-1, Rel A, IL-5, TNF- α , p210^{bcr-abl} or RSV encoding mRNA is reduced below that observed in the absence of the ribozyme, and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the level of ICAM-1, IL-5, Rel A, TNF- α , p210^{bcr-abl} or RSV protein or activity in a cell or tissue. By "related" is meant that the inhibition of ICAM-1, IL-5, Rel A, TNF- α , p210^{bcr-abl} or RSV mRNA translation, and thus reduction in the level of, ICAM-1, IL-5, Rel A, TNF- α , p210^{bcr-abl} or RSV proteins will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues through the use of a catheter, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables 2,3,6-9, 11, 13, 15-23, 27, 28, 31, 33, 34, 36 and 37.

Examples of such ribozymes are shown in Tables 4-8, 10, 12, 14-16, 19-22, 24, 26-28, 30, 32, 34 and 36-38. Examples of such ribozymes consist essentially of sequences defined in these Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in the above identified Tables can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in the above identified Tables can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequence listed in the above identified Tables may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit ICAM-1, IL-5, Rel A, TNF- α , p210^{bcr-abl} or RSV gene expression are expressed from transcription units inserted into DNA, RNA, or viral vectors. Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA or RNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 *Proc. Natl. Acad. Sci. USA*, 87, 6743-7; Gao and Huang 1993 *Nucleic Acids Res.*, 21 2867-72; Lieber et al., 1993 *Methods Enzymol.*, 217, 47-66; Zhou et al., 1990 *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 *Antisense Res. Dev.*, 2, 3-15; Ojwang et al., 1992 *Proc. Natl. Acad. Sci. USA*, 90, 6340-4; L'Huillier et al., 1992 *EMBO J.* 11, 4411-8; Lisiewicz et al., 1993 *Proc. Natl. Acad. Sci. U.S.A.*, 90 8000-4). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors

(such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors).

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from
5 the claims.

Description Of The Preferred Embodiments

The drawings will first briefly be described.

Drawings:

Figure 1 is a diagrammatic representation of the hammerhead
10 ribozyme domain known in the art. Stem II can be ≥ 2 base-pair long.

Figure 2(a) is a diagrammatic representation of the hammerhead
ribozyme domain known in the art; Figure 2(b) is a diagrammatic
representation of the hammerhead ribozyme as divided by Uhlenbeck
(1987, *Nature*, 327, 596-600) into a substrate and enzyme portion; Figure
15 2(c) is a similar diagram showing the hammerhead divided by Haseloff and
Gerlach (1988, *Nature*, 334, 585-591) into two portions; and Figure 2(d) is
a similar diagram showing the hammerhead divided by Jeffries and
Symons (1989, *Nucl. Acids. Res.*, 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the general structure of a
20 hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, *n*
is 1,2,3 or 4) and helix 5 can be optionally provided of length 2 or more
bases (preferably 3-20 bases, *i.e.*, *m* is from 1-20 or more). Helix 2 and
helix 5 may be covalently linked by one or more bases (*i.e.*, *r* is ≥ 1 base).
Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4-20
25 base pairs) to stabilize the ribozyme structure, and preferably is a protein
binding site. In each instance, each N and N' independently is any normal
or modified base and each dash represents a potential base-pairing
interaction. These nucleotides may be modified at the sugar, base or
phosphate. Complete base-pairing is not required in the helices, but is
30 preferred. Helix 1 and 4 can be of any size (*i.e.*, *o* and *p* is each
independently from 0 to any number, *e.g.* 20) as long as some base-pairing
is maintained. Essential bases are shown as specific bases in the
structure, but those in the art will recognize that one or more may be

modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, *i.e.*, without a connecting loop. The connecting loop when present may be a ribonucleotide with or without
5 modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases. "____" refers to a covalent bond.

Figure 4 is a representation of the general structure of the hepatitis
10 delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 is a diagrammatic representation of the genetic map of RSV strain A2.

15 Figure 7 is a diagrammatic representation of the solid-phase synthesis of RNA.

Figure 8 is a diagrammatic representation of exocyclic amino protecting groups for nucleic acid synthesis.

Figure 9 is a diagrammatic representation of the deprotection of RNA.

20 Figure 10 is a graphical representation of the cleavage of an RNA substrate by ribozymes synthesized, deprotected and purified using the improved methods described herein.

Figure 11 is a schematic representation of a two pot deprotection protocol. Base deprotection is carried out with aqueous methyl amine at 65
25 °C for 10 min. The sample is dried in a speed-vac for 2-24 hours depending on the scale of RNA synthesis. Silyl protecting group at the 2'-hydroxyl position is removed by treating the sample with 1.4 M anhydrous HF at 65°C for 1.5 hours.

30 Figure 12 is a schematic representation of a one pot deprotection of RNA synthesized using RNA phosphoramidite chemistry. Anhydrous methyl amine is used to deprotect bases at 65°C for 15 min. The sample is allowed to cool for 10 min before adding TEA•3HF reagent, to the same

pot, to remove protecting groups at the 2'-hydroxyl position. The deprotection is carried out for 1.5 hours.

Figs. 13a - b is a HPLC profile of a 36 nt long ribozyme, targeted to site B. The RNA is deprotected using either the two pot or the one pot deprotection protocol. The peaks corresponding to full-length RNA is indicated. The sequence for site B is CCUGGGCCAGGGAUUA AUGGAGAUGCCCACU.

Figure 14 is a graph comparing RNA cleavage activity of ribozymes deprotected by two pot vs one pot deprotection protocols.

Figure 15 is a schematic representation of an improved method of synthesizing RNA containing phosphorothioate linkages.

Figure 16 shows RNA cleavage reaction catalyzed by ribozymes containing phosphorothioate linkages. Hammerhead ribozyme targeted to site C is synthesized such that 4 nts at the 5' end contain phosphorothioate linkages. P=O refers to ribozyme without phosphorothioate linkages. P=S refers to ribozyme with phosphorothioate linkages. The sequence for site C is UCAUUUUGGCAUCUC UCCUUCAGGCGUGG.

Figure 17 is a schematic representation of synthesis of 2'-N-phtalimido-nucleoside phosphoramidite.

Figure 18 is a diagrammatic representation of a prior art method for the solid-phase synthesis of RNA using silyl ethers, and the method of this invention using SEM as a 2'-protecting group.

Figure 19 is a diagrammatic representation of the synthesis of 2'-SEM-protected nucleosides and phosphoramidites useful for the synthesis of RNA. B is any nucleotide base as exemplified in the Figure, P is purine and I is inosine. Standard abbreviations are used throughout this application, well known to those in the art.

Figure 20 is a diagrammatic representation of a prior art method for deprotection of RNA using TBDMS protection of the 2'-hydroxyl group.

Figure 21 is a diagrammatic representation of the deprotection of RNA having SEM protection of the 2'-hydroxyl group.

Figure 22 is a representation of an HPLC chromatogram of a fully deprotected 10-mer of uridylic acid.

Figs. 23 - 25 are diagrammatic representations of hammerhead, hairpin or hepatitis delta virus ribozyme containing self-processing RNA transcript. Solid arrows indicate self-processing sites. Boxes indicate the sites of nucleotide substitution. Solid lines are drawn to show the binding sites of primers used in a primer-extension assay. Lower case letters indicate vector sequence present in the RNA when transcribed from a *HindIII*-linearized plasmid. (23) HH Cassette, transcript containing the hammerhead trans-acting ribozyme linked to a 3' cis-acting hammerhead ribozyme. The structure of the hammerhead ribozyme is based on phylogenetic and mutational analysis (reviewed by Symons, 1992 *supra*). The trans ribozyme domain extends from nucleotide 1 through 49. After 3'-end processing, the trans-ribozyme contains 2 non-ribozyme nucleotides (UC at positions 50 and 51) at its 3' end. The 3' processing ribozyme is comprised of nucleotides 44 through 96. Roman numerals I, II and III, indicate the three helices that contribute to the structure of the 3' cis-acting hammerhead ribozyme (Hertel et al., 1992 *Nucleic Acids Res.* 20, 3252). Substitution of G70 and A71 to U and G respectively, inactivates the hammerhead ribozyme (Ruffner et al., 1990 *Biochemistry* 29, 10695) and generates the HH(mutant) construct. (24) HP Cassette, transcript containing the hammerhead trans-acting ribozyme linked to a 3' cis-acting hairpin ribozyme. The structure of the hairpin ribozyme is based on phylogenetic and mutational analysis (Berzal-Herranz et al., 1993 *EMBO J* 12, 2567). The trans-ribozyme domain extends from nucleotide 1 through 49. After 3'-end processing, the trans-ribozyme contains 5 non-ribozyme nucleotides (UGGCA at positions 50 to 54) at its 3' end. The 3' cis-acting ribozyme is comprised of nucleotides 50 through 115. The transcript named HP(GU) was constructed with a potential wobble base pair between G52 and U77; HP(GC) has a Watson-Crick base pair between G52 and C77. A shortened helix 1 (5 base pairs) and a stable tetraloop (GAAA) at the end of helix 1 was used to connect the substrate with the catalytic domain of the hairpin ribozyme (Feldstein & Bruening, 1993 *Nucleic Acids Res.* 21, 1991; Altschuler et al., 1992 *supra*). (25) HDV Cassette, transcript containing the trans-acting hammerhead ribozyme linked to a 3' cis-acting hepatitis delta virus (HDV) ribozyme. The secondary structure of the HDV ribozyme is as proposed by Been and

coworkers (Been et al., 1992 Biochemistry 31, 11843). The trans-ribozyme domain extends from nucleotides 1 through 48. After 3'-end processing, the trans-ribozyme contains 2 non-ribozyme nucleotides (AA at positions 49 to 50) at its 3' end. The 3' cis-acting HDV ribozyme is comprised of nucleotides 50 through 114. Roman numerals I, II, III & IV, indicate the location of four helices within the 3' cis-acting HDV ribozyme (Perrota & Been, 1991 Nature 350, 434). The Δ HDV transcript contains a 31 nucleotide deletion in the HDV portion of the transcript (nucleotides 84 through 115 deleted).

- 10 Fig. 26 is a schematic representation of a plasmid containing the insert encoding self-processing cassette. The figure is not drawn to scale.

Fig. 27 demonstrates the effect of 3' flanking sequences on RNA self-processing *in vitro*. H, Plasmid templates linearized with *Hind*III restriction enzyme. Transcripts from H templates contain four non-ribozyme nucleotides at the 3' end. N, Plasmid templates linearized with *Nde*I restriction enzyme. Transcripts from N templates contain 220 non-ribozyme nucleotides at the 3' end. R, Plasmid templates linearized with *Rca*I restriction enzyme. Transcripts from R templates contain 450 non-ribozyme nucleotides at the 3' end.

- 20 Fig. 28 shows the effect of 3' flanking sequences on the trans-cleavage reaction catalyzed by a hammerhead ribozyme. A 622 nt internally-labeled RNA (<10 nM) was incubated with ribozyme (1000 nM) under single turn-over conditions (Herschlag and Cech, 1990 Biochemistry 29, 10159). HH+2, HH+37, and HH+52 are trans-acting ribozymes produced by transcription from the HH, Δ HDV, and HH(mutant) constructs, respectively, and that contain 2, 37 and 52 extra nucleotides on the 3' end. The plot of the fraction of uncleaved substrate versus time was fit to a double exponential curve using the KaleidaGraph graphing program (Synergy Software, Reading, PA). A double exponential curve fit was used because the data points did not fall on a single exponential curve, presumably due to varying conformers of ribozyme and/or substrate RNA.

Fig. 29 shows RNA self-processing in OST7-1 cells. *In vitro* lanes contain full-length, unprocessed transcripts that were added to cellular lysates prior to RNA extraction. These RNAs were either pre-incubated with $MgCl_2$ (+) or with DEPC-treated water (-) prior to being hybridized

with 5' end-labeled primers. Cellular lanes contain total cellular RNA from cells transfected with one of the four self-processing constructs. Cellular RNA are probed for ribozyme expression using a sequence specific primer-extension assay. Solid arrows indicate the location of primer extension
5 bands corresponding to Full-Length RNA and 3' Cleavage Products.

Figs. 30,31 are diagrammatic representations of self-processing cassettes that will release trans-acting ribozymes with defined, stable stem-loop structures at the 5' and the 3' end following self-processing. 30, shows various permutations of a hammerhead self-processing cassette. 31,
10 shows various permutations of a hairpin self-processing cassette.

Figs. 32a-b Schematic representation of RNA polymerase III promoter structure. Arrow indicates the transcription start site and the direction of coding region. A, B and C, refer to consensus A, B and C box promoter sequences. I, refers to intermediate cis-acting promoter sequence. PSE,
15 refers to proximal sequence element. DSE, refers to distal sequence element. ATF, refers to activating transcription factor binding element. ?, refers to cis-acting sequence element that has not been fully characterized. EBER, Epstein-Barr-virus-encoded-RNA. TATA is a box well known in the art.

20 Figs. 33a-e Sequence of the primary tRNAⁱ_{met} and Δ3-5 transcripts. The A and B box are internal promoter regions necessary for pol III transcription. Arrows indicate the sites of endogenous tRNA processing. The Δ3-5 transcript is a truncated version of tRNA wherein the sequence 3' of B box has been deleted (Adeniyi-Jones et al., 1984 *supra*). This
25 modification renders the Δ 3-5 RNA resistant to endogenous tRNA processing.

Figure 34. Schematic representation of RNA structural motifs inserted into the Δ3-5 RNA. Δ3-5/HHI- a hammerhead (HHI) ribozyme was cloned at the 3' region of Δ3-5 RNA; S3- a stable stem-loop structure was
30 incorporated at the 3' end of the Δ3-5/HHI chimera; S5- stable stem-loop structures were incorporated at the 5' and the 3' ends of Δ3-5/HHI ribozyme chimera; S35- sequence at the 3' end of the Δ3-5/HHI ribozyme chimera was altered to enable duplex formation between the 5' end and a complementary 3' region of the same RNA; S35Plus- in addition to
35 structural alterations of S35, sequences were altered to facilitate additional

duplex formation within the non-ribozyme sequence of the $\Delta 3$ -5/HHI chimera.

Figures 35 and 36. Northern analysis to quantitate ribozyme expression in T cell lines transduced with $\Delta 3$ -5 vectors. 35) $\Delta 3$ -5/HHI and its variants were cloned individually into the DC retroviral vector (Sullenger et al., 1990 *supra*). Northern analysis of ribozyme chimeras expressed in MT-2 cells was performed. Total RNA was isolated from cells (Chomczynski & Sacchi, 1987 *Analytical Biochemistry* 162, 156-159), and transduced with various constructs described in Fig. 34. Northern analysis was carried out using standard protocols (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). Nomenclature is same as in Figure 34. This assay measures the level of expression from the type 2 pol III promoter. 36) Expression of S35 constructs in MT2 cells. S35 (+ribozyme), S35 construct containing HHI ribozyme. S35 (-ribozyme), S35 construct containing no ribozyme.

Figure 37. Ribozyme activity in total RNA extracted from transduced MT-2 cells. Total RNA was isolated from cells transduced with $\Delta 3$ -5 constructs described in Figs. 35 and 36. In a standard ribozyme cleavage reaction, 5 μ g total RNA and trace amounts of 5' terminus-labeled ribozyme target RNA were denatured separately by heating to 90°C for 2 min in the presence of 50 mM Tris-HCl, pH 7.5 and 10 mM MgCl₂. RNAs were renatured by cooling the reaction mixture to 37°C for 10-15 min. Cleavage reaction was initiated by mixing the labeled substrate RNA and total cellular RNA at 37°C. The reaction was allowed to proceed for ~ 18h, following which the samples were resolved on a 20 % urea-polyacrylamide gel. Bands were visualized by autoradiography.

Figures 38 and 39. Ribozyme expression and activity levels in S35-transduced clonal CEM cell lines. 38) Northern analysis of S35-transduced clonal CEM cell lines. Standard curve was generated by spiking known concentrations of in vitro transcribed S5 RNA into total cellular RNA isolated from non-transduced CEM cells. Pool, contains RNA from pooled cells transduced with S35 construct. Pool (-G418 for 3 Mo), contains RNA from pooled cells that were initially selected for resistance to G418 and then grown in the absence of G418 for 3 months. Lanes A through N contain RNA from individual clones that were generated from the pooled cells transduced with S35 construct. tRNA_i^{met}, refers to the

endogenous tRNA. S35, refers to the position of the ribozyme band. M, marker lane. 39) Activity levels in S35-transduced clonal CEM cell lines. RNA isolation and cleavage reactions were as described in Fig.37. Nomenclature is same as in Figs. 35 and 36 except, S, 5' terminus-labeled substrate RNA. P, 8 nt 5' terminus-labeled ribozyme-mediated RNA cleavage product.

Figures 40 and 41 are proposed secondary structures of S35 and S35 containing a desired RNA (HHI), respectively. The position of HHI ribozyme is indicated in figure 41. Intramolecular stem refers to the stem structure formed due to an intramolecular base-paired interaction between the 3' sequence and the complementary 5' terminus. The length of the stem ranges from 15-16 base-pairs. Location of the A and the B boxes are shown.

Figures 42 and 43 are proposed secondary structures of S35 plus and S35 plus containing HHI ribozyme.

Figures 44, 45, 46 and 47 are the nucleotide base sequences of S35, HHIS35, S35 Plus, and HHIS35 Plus respectively.

Figs. 48a-b is a general formula for pol III RNA of this invention.

Figure 49 is a digrammatic representation of 5T construct. In this construct the desired RNA is located 3' of the intramolecular stem.

Figures 50 and 51 contain proposed secondary structures of 5T construct alone and 5T construct containing a desired RNA (HHI ribozyme) respectively.

Figure 52 is a diagrammatic representation of TRZ-tRNA chimeras. The site of desired RNA insertion is indicated.

Figure 53 shows the general structure of HHITRZ-A ribozyme chimera. A hammerhead ribozyme targeted to site I is inserted into the stem II region of TRZ-tRNA chimera.

Figure 54 shows the general structure of HPITRZ-A ribozyme chimera. A hairpin ribozyme targeted to site I is cloned into the indicated region of TRZ-tRNA chimera.

Figure 55 shows a comparison of RNA cleavage activity of HHITRZ-A, HHITRZ-B and a chemically synthesized HH hammerhead ribozymes.

Figure 56 shows expression of ribozymes in T cell lines that are stably transduced with viral vectors. M, markers; lane 1, non-transduced CEM
5 cells; lanes 2 and 3, MT2 and CEM cells transduced with retroviral vectors; lanes 4 and 5, MT2 and CEM cells transduced with AAV vectors.

Figs. 57a-b Schematic diagram of adeno-associated virus and
adenoviruses vectors for ribozyme delivery. Both vectors utilize one or more
ribozyme encoding transcription units (RZ) based on RNA polymerase II or
10 RNA polymerase III promoters. A. Diagram of an AAV-based vector
containing minimal AAV sequences comprising the inverted terminal
repeats (ITR) at each end of the vector genome, an optional selectable
marker (Neo) driven by an exogenous promoter (Pro), a ribozyme
transcription unit, and sufficient additional sequences (stuffer) to maintain a
15 vector length suitable for efficient packaging. B. Diagram of ribozyme
expressing adenovirus vectors containing deletions of one or more wild
type adenovirus coding regions (cross-hatched boxes marked as E1, pIX,
E3, and E4), and insertion of the ribozyme transcription unit at any or
several of those regions of deletions.

20 Fig. 58 is a graph showing the effect of arm length variation on the
activity of ligated hammerhead (HH) ribozymes. Nomenclature 5/5, 6/6,
7/7, 8/8 and so on refers to the number of base-pairs being formed between
the ribozyme and the target. For example, 5/8 means that the HH ribozyme
forms 5 bp on the 5' side and 8 bp on the 3' side of the cleavage site for a
25 total of 13 bp. $-\Delta G$ refers to the free energy of binding calculated for base-
paired interactions between the ribozyme and the substrate RNA (Turner
and Sugimoto, 1988 Ann. Rev. Biophys. Chem. 17, 167). RPI A is a HH
ribozyme with 6/6 binding arms.

30 Figs. 59 and 60 and 61 show cleavage of long substrate (622 nt) by
ligated HH ribozymes.

Fig. 62 is a diagrammatic representation of a hammerhead ribozyme
(HH-H) targeted against a site termed H. Variants of HH-H are also shown
that contain either a 2 base-paired stem II (HH-H1 and HH-H2) or a 3 base-
paired stem II (HH-H3 and HH-H4).

Figs. 63 and 64 show RNA cleavage activity of HH-I and its variants (see Fig.62). 63) cleavage of matched substrate RNA (15 nt). 64) cleavage of long substrate RNA (613 nt).

5 Figs. 65a-b is a schematic representation of a method of this invention to synthesize a full length hairpin ribozyme. No splint strand is required for ligation but rather the two fragments hybridize together at helix 4 prior to ligation. The only prerequisite is that the 3' fragment is phosphorylated at its 5' end and that the 3' end of the 5' fragment have a hydroxyl group. The hairpin ribozyme is targeted against site J. H1 and H2 are intermolecular
10 helices formed between the ribozyme and the substrate. H3 and H4 are intramolecular helices formed within the hairpin ribozyme motif. Arrow indicates the cleavage site.

Fig. 66 shows RNA cleavage activity of ligated hairpin ribozymes targeted against site J.

15 Figs. 67a-b is a diagrammatic representation of a Site K Hairpin Ribozyme (HP-K) showing the proposed secondary structure of the hairpin ribozyme •substrate complex as described in the art (Berzal-Herranz *et al.*, 1993 *EMBO. J.*12, 2567). The ribozyme has been assembled from two fragments (bimolecular ribozyme; Chowrira and Burke, 1992 *Nucleic Acids*
20 *Res.* 20, 2835); #H1 and H2 represent intermolecular helix formation between the ribozyme and the substrate. H3 and H4 represent intramolecular helix formation within the ribozyme (intermolecular helix in the case of bimolecular ribozyme). Left panel (HP-K1) indicates 4 base-paired helix 2 and the right panel (HP-K2) indicates 6 base-paired helix 2.
25 Arrow indicates the site of RNA cleavage. All the ribozymes discussed herein were chemically synthesized by solid phase synthesis using RNA phosphoramidite chemistry, unless otherwise indicated. Those skilled in the art will recognize that these ribozymes could also be made transcriptionally *in vitro* and *in vivo*.

30 Figure 68 is a graph showing RNA cleavage by hairpin ribozymes targeted to site K. A plot of fraction of the target RNA uncleaved (fraction uncleaved) as a function of time is shown. HP-K2 (6 bp helix 2) cleaves a 422 target RNA to a greater extent than the HP-K1 (4 bp helix 2).

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 422 nt region (containing hairpin site A) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed in a standard transcription buffer in the presence of [α -³²P]CTP (Chowrira & Burke, 1991 *supra*). The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (1 μ M) and internally labeled 422 nt substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Figs. 69a-b is the Site L Hairpin Ribozyme (HP-L) showing proposed secondary structure of the hairpin ribozyme-substrate complex. The ribozyme was assembled from two fragments as described above. The nomenclature is the same as above.

Figure 70 shows RNA cleavage by hairpin ribozymes targeted to site L. A. plot of fraction of the target RNA uncleaved (fraction uncleaved) as a function of time is shown. HP-L2 (6 bp helix 2) cleaves a 2 KB target RNA to a greater extent than the HP-L1 (4 bp helix 2). To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 2 kb region (containing hairpin site L) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. The cleavage reactions were carried out as described above.

Figs. 71a-b shows a Site M Hairpin Ribozyme (HP-M) with the proposed secondary structure of the hairpin ribozyme-substrate complex. The ribozyme was assembled from two fragments as described above.

Figure 72 is a graph showing RNA cleavage by hairpin ribozymes targeted to site M. The ribozymes were tested at both 20°C and at 26°C. To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 1.9 KB region (containing hairpin site M) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Cleavage reactions were carried out as described above except that 20°C and at 26°C temperatures were used.

Figs. 73a-d shows various structural modifications of the present invention. A) Hairpin ribozyme lacking helix 5. Nomenclature is same as described under figure 3. B) Hairpin ribozyme lacking helix 4 and helix 5. Helix 4 is replaced by a nucleotide loop wherein q is ≥ 2 bases. Nomenclature is same as described under figure 3. C) Hairpin ribozyme lacking helix 5. Helix 4 loop is replaced by a linker 10^3 "L", wherein L is a non-nucleotide linker molecule (Benseler *et al.*, 1993 *J. Am. Chem. Soc.* 115, 8483; Jennings *et al.*, WO 94/13688). Nomenclature is same as described under figure 3. D) Hairpin ribozyme lacking helix 4 and helix 5. Helix 4 is replaced by non-nucleotide linker molecule "L" (Benseler *et al.*, 1993 *supra*; Jennings *et al.*, *supra*). Nomenclature is same as described under figure 3.

Figs. 74a-b shows Hairpin ribozymes containing nucleotide spacer region "s" at the indicated location, wherein s is ≥ 1 base. Hairpin ribozymes containing spacer region, can be synthesized as one fragment or can be assembled from multiple fragments. Nomenclature is same as described under figure 3.

Figs. 75a-e shows the structures of the 5'-C-alkyl-modified nucleotides. R_1 is as defined above. R is OH, H, O-protecting group, NH, or any group described by the publications discussed above, and those described below. B is as defined in the Figure or any other equivalent nucleotide base. CE is cyanoethyl, DMT is a standard blocking group. Other abbreviations are standard in the art.

Figure 76 is a diagrammatic representation of the synthesis of 5'-C-alkyl-D-allose nucleosides and their phosphoramidites.

Figure 77 is a diagrammatic representation of the synthesis of 5'-C-alkyl-L-talose nucleosides and their phosphoramidites.

- 5 Figure 78 is a diagrammatic representation of hammerhead ribozymes targeted to site O containing 5'-C-methyl-L-talo modifications at various positions.

Figure 79 shows RNA cleavage activity of HH-O ribozymes. Fraction of target RNA uncleaved as a function of time is shown.

- 10 Figure 80 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al. Nucleic Acids Res.* 1992, 20, 3252) showing specific substitutions.

- 15 Figs. 81a-j shows the structures of various 2'-alkyl modified nucleotides which exemplify those of this invention. R groups are alkyl groups, Z is a protecting group.

Figure 82 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine.

Figure 83 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene uridine.

- 20 Figure 84 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene cytidine.

Figure 85 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene adenosine.

- 25 Figure 86 is a diagrammatic representation of the synthesis of 2'-C-carboxymethylidene uridine, 2'-C-methoxycarboxymethylidene uridine and derivatized amidites thereof. X is CH₃ or alkyl as discussed above, or another substituent.

Figure 87 is a diagrammatic representation of a synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonates.

Figure 88 is a diagrammatic representation of the synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonate 3'-phosphoramidites, dimers and solid supported dimers.

Figure 89 is a diagrammatic representation of the synthesis of
5 nucleoside 5'-deoxy-5'-difluoromethylene triphosphates.

Figures 90 and 91 are diagrammatic representations of the synthesis of 3'-deoxy-3'-difluoromethylphosphonates and dimers.

Figure 92 is a schematic representation of synthesizing RNA phosphoramidite of a nucleotide containing a 2'-hydroxyl group
10 modification of the present invention.

Figs. 93a-b describes a method for deprotection of oligonucleotides containing a 2'-hydroxyl group modification of the present invention.

Figure 94 is a diagrammatic representation of a hammerhead ribozyme targeted to site N. Positions of 2'-hydroxyl group substitution is
15 indicated.

Figure 95 shows RNA cleavage activity of ribozymes containing a 2'-hydroxyl group modification of the present invention. All RNA, represents hammerhead ribozyme (HHN) with no 2'-hydroxyl group modifications. U7-ala, represents HHN ribozyme containing 2'-NH-alanine modification at the
20 U7 position. U4/U7-ala, represents HHA containing 2'-NH-alanine modifications at U4 and U7 positions. U4 lys, represents HHA containing 2'-NH-lysine modification at U4 position. U7 lys, represents HHA containing 2'-NH-lysine modification at U7 position. U4/U7-lys, represents HHN containing 2'-NH-lysine modification at U4 and U7 positions.

25 Figures 96 and 97 are schematic representations of synthesizing (solid-phase synthesis) 3' ends of RNA with modification of the present invention. B, refers to either a base, modified base or an H.

Figure 98 and 99 are schematic representations of synthesizing (solid-phase synthesis) 5' ends of RNA with modification of the present
30 invention. B, refers to either a base, modified base or an H.

Figures 100 and 101 are general schematic representations of the invention.

Fig. 102a-d is a schematic representation of a method of the invention.

Fig. 103 is a graph of the results of the experiment diagrammed in figure 104.

Figure 104 is a diagrammatic representation of a fusion mRNA used
5 in the experiment diagrammed in Fig. 102.

Figure 105 is a diagrammatic representation of a method for selection of useful ribozymes of this invention.

Figure 106 generally shows R-loop formation, and an R-loop complex. In addition, it indicates the location at which ligands can be
10 provided to target the R-loop complex to cells using at least three different procedures, such as ligand receptor interaction, lipid or calcium phosphate mediated delivery, or electroporation.

Figure 107 shows a method for use of self-processing ribozymes to generate therapeutic ribozymes of unit length. This method is essentially
15 described by Draper et al., PCT WO 93/23509.

Figure 108 shows a method of linking ligands like folate, carbohydrate or peptides to R-loop forming RNA.

Ribozymes of this invention block to some extent ICAM-1, IL-5, rel A, TNF- α , p210^{bcr-abl}, or RSV genes expression and can be used to treat
20 diseases or diagnose such diseases. Ribozymes will be delivered to cells in culture and to tissues in animal models. Ribozyme cleavage of ICAM-1, IL-5, rel A, TNF- α , p210^{bcr-abl}, or RSV mRNA in these systems may prevent or alleviate disease symptoms or conditions.

I. Target sites

25 Targets for useful ribozymes can be determined as disclosed in Draper et al PCT WO93/23509, Sullivan *et al.*, PCT WO94/02595 as well as by Draper et al., PCT/US94/13129 and hereby incorporated by reference herein in totality. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such
30 methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. Such ribozymes can also be

optimized and delivered as described therein. While specific examples to animal and human RNA are provided, those in the art will recognize that the equivalent human RNA targets described can be used as described below. Thus, the same target may be used, but binding arms suitable for
5 targeting human RNA sequences are present in the ribozyme. Such targets may also be selected as described below.

It must be established that the sites predicted by the computer-based RNA folding algorithm correspond to potential cleavage sites. Hammerhead or hairpin ribozymes are designed that could bind and are
10 individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci., USA, 86 7706-7710) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm
15 lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

mRNA is screened for accessible cleavage sites by the method described generally in Draper et al., PCT WO93/23569 hereby incorporated by reference herein. Briefly, DNA oligonucleotides
20 representing potential hammerhead or hairpin ribozyme cleavage sites are synthesized. A polymerase chain reaction is used to generate a substrate for T7 RNA polymerase transcription from cDNA clones. Labeled RNA transcripts are synthesized *in vitro* from DNA templates. The oligonucleotides and the labeled transcripts are annealed, RNaseH is
25 added and the mixtures are incubated for the designated times at 37°C. Reactions are stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved is determined by autoradiographic quantitation using a phosphor imaging system. From these data, hammerhead or hairpin ribozyme sites are chosen as the
30 most accessible.

Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used
35 follows the procedure for normal RNA synthesis as described in Usman et al., 1987 *J. Am. Chem. Soc.*, 109, 7845 and in Scaringe et al., 1990

Nucleic Acids Res., 18, 5433 and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, phosphoramidites at the 3'-end. The average stepwise coupling yields are >98%. Inactive ribozymes are synthesized by substituting a U for G₅ and a U for A₁₄ (numbering from Hertel et al., 1992 *Nucleic Acids Res.*, 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840). Ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbach, 1989, *Methods Enzymol*, 180, 51). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992 *TIBS* 17,34). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography and are resuspended in water.

Example 1: ICAM-1

Ribozymes that cleave ICAM-1 mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders. ICAM-1 function can be blocked therapeutically using monoclonal antibodies. Ribozymes have the advantage of being generally immunologically inert, whereas significant neutralizing anti-IgG responses can be observed with some monoclonal antibody treatments.

The following is a brief description of the physiological role of ICAM-1. The discussion is not meant to be complete and is provided only for understanding of the invention that follows. This summary is not an admission that any of the work described below is prior art to the claimed invention.

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface protein whose expression is induced by inflammatory mediators. ICAM-1 is required for adhesion of leukocytes to endothelial cells and for several immunological functions including antigen presentation, immunoglobulin production and cytotoxic cell activity. Blocking ICAM-1 function prevents immune cell recognition and activity during transplant rejection and in animal models of rheumatoid arthritis, asthma and reperfusion injury.

- Cell-cell adhesion plays a pivotal role in inflammatory and immune responses (Springer et al., 1987 *Ann. Rev. Immunol.* 5, 223-252). Cell adhesion is required for leukocytes to bind to and migrate through vascular endothelial cells. In addition, cell-cell adhesion is required for antigen presentation to T cells, for B cell induction by T cells, as well as for the cytotoxicity activity of T cells, NK cells, monocytes or granulocytes. Intercellular adhesion molecule-1 (ICAM-1) is a 110 kilodalton member of the immunoglobulin superfamily that is involved in all of these cell-cell interactions (Simmons et al., 1988 *Nature (London)* 331, 624-627).
- 10 ICAM-1 is expressed on only a limited number of cells and at low levels in the absence of stimulation (Dustin et al., 1986 *J. Immunol.* 137, 245-254). Upon treatment with a number of inflammatory mediators (lipopolysaccharide, γ -interferon, tumor necrosis factor- α , or interleukin-1), a variety of cell types (endothelial, epithelial, fibroblastic and hematopoietic cells) in a variety of tissues express high levels of ICAM-1 on their surface (Springer et al. *supra*; Dustin et al., *supra*; and Rothlein et al., 1988 *J. Immunol.* 141, 1665-1669). Induction occurs via increased transcription of ICAM-1 mRNA (Simmons et al., *supra*). Elevated expression is detectable after 4 hours and peaks after 16 - 24 hours of induction.
- 20 ICAM-1 induction is critical for a number of inflammatory and immune responses. *In vitro*, antibodies to ICAM-1 block adhesion of leukocytes to cytokine-activated endothelial cells (Boyd, 1988 *Proc. Natl. Acad. Sci. USA* 85, 3095-3099; Dustin and Springer, 1988 *J. Cell Biol.* 107, 321-331). Thus, ICAM-1 expression may be required for the extravasation of immune cells to sites of inflammation. Antibodies to ICAM-1 also block T cell killing, mixed lymphocyte reactions, and T cell-mediated B cell differentiation, suggesting that ICAM-1 is required for these cognate cell interactions (Boyd et al., *supra*). The importance of ICAM-1 in antigen presentation is underscored by the inability of ICAM-1 defective murine B cell mutants to stimulate antigen-dependent T cell proliferation (Dang et al., 1990 *J. Immunol.* 144, 4082-4091). Conversely, murine L cells require transfection with human ICAM-1 in addition to HLA-DR in order to present antigen to human T cells (Altmann et al., 1989 *Nature (London)* 338, 512-514). In summary, evidence *in vitro* indicates that ICAM-1 is required for cell-cell interactions critical to inflammatory responses, cellular immune responses, and humoral antibody responses.
- 35

By engineering ribozyme motifs we have designed several ribozymes directed against ICAM-1 mRNA sequences. These have been synthesized with modifications that improve their nuclease resistance. These ribozymes cleave ICAM-1 target sequences *in vitro*.

5 The sequence of human, rat and mouse ICAM-1 mRNA can be screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and that contain potential hammerhead or hairpin ribozyme cleavage sites can be identified. These sites are shown in Tables 2, 3, and 6-9. (All sequences
10 are 5' to 3' in the tables) While rat, mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility.

15 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 4 - 8 and 10. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity and may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

20 The ribozymes will be tested for function *in vivo* by exogenous delivery to human umbilical vein endothelial cells (HUVEC). Ribozymes will be delivered by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA or RNA vectors described above. Cytokine-induced ICAM-1 expression will be
25 monitored by ELISA, by indirect immunofluoresence, and/or by FACS analysis. ICAM-1 mRNA levels will be assessed by Northern, by RNase protection, by primer extension or by quantitative RT-PCR analysis. Ribozymes that block the induction of ICAM-1 protein and mRNA by more than 90% will be identified.

30 As disclosed by Sullivan et al., PCT WO94/02595, incorporated by reference herein, ribozymes and/or genes encoding them will be locally delivered to transplant tissue *ex vivo* in animal models. Expression of the ribozyme will be monitored by its ability to block *ex vivo* induction of ICAM-1 mRNA and protein. The effect of the anti-ICAM-1 ribozymes on graft
35 rejection will then be assessed. Similarly, ribozymes will be introduced

into joints of mice with collagen-induced arthritis or rabbits with *Streptococcal* cell wall-induced arthritis. Liposome delivery, cationic lipid delivery, or adeno-associated virus vector delivery can be used. One dose (or a few infrequent doses) of a stable anti-ICAM-1 ribozyme or a gene
5 construct that constitutively expresses the ribozyme may abrogate inflammatory and immune responses in these diseases.

Uses

ICAM-1 plays a central role in immune cell recognition and function. Ribozyme inhibition of ICAM-1 expression can reduce transplant rejection
10 and alleviate symptoms in patients with rheumatoid arthritis, asthma or other acute and chronic inflammatory disorders. We have engineered several ribozymes that cleave ICAM-1 mRNA. Ribozymes that efficiently inhibit ICAM-1 expression in cells can be readily found and their activity measured with regard to their ability to block transplant rejection and
15 arthritis symptoms in animal models. These anti-ICAM-1 ribozymes represent a novel therapeutic for the treatment of immunological or inflammatory disorders.

The therapeutic utility of reduction of activity of ICAM-1 function is evident in the following disease targets. The noted references indicate the
20 role of ICAM-1 and the therapeutic potential of ribozymes described herein. Thus, these targets can be therapeutically treated with agents that reduce ICAM-1 expression or function. These diseases and the studies that support a critical role for ICAM-1 in their pathology are listed below. This list is not meant to be complete and those in the art will recognize further
25 conditions and diseases that can be effectively treated using ribozymes of the present invention.

- Transplant rejection

ICAM-1 is expressed on venules and capillaries of human cardiac biopsies with histological evidence of graft rejection (Briscoe et al., 1991 *Transplantation*
30 51, 537-539).

Antibody to ICAM-1 blocks renal (Cosimi et al., 1990 *J. Immunol.* 144, 4604-4612) and cardiac (Flavin et al., 1991 *Transplant. Proc.* 23, 533-534) graft rejection in primates.

A Phase I clinical trial of a monoclonal anti-ICAM-1 antibody showed significant reduction in rejection and a significant increase in graft function in human kidney transplant patients (Haug, et al., 1993 *Transplantation* 55, 766-72).

- Rheumatoid arthritis

- 5 ICAM-1 overexpression is seen on synovial fibroblasts, endothelial cells, macrophages, and some lymphocytes (Chin et al., 1990 *Arthritis Rheum* 33, 1776-86; Koch et al., 1991 *Lab Invest* 64, 313-20).

Soluble ICAM-1 levels correlate with disease severity (Mason et al., 1993 *Arthritis Rheum* 36, 519-27).

- 10 Anti-ICAM antibody inhibits collagen-induced arthritis in mice (Kakimoto et al., 1992 *Cell Immunol* 142, 326-37).

Anti-ICAM antibody inhibits adjuvant-induced arthritis in rats (Iigo et al., 1991 *J Immunol* 147, 4167-71).

- Myocardial ischemia, stroke, and reperfusion injury

- 15 Anti-ICAM-1 antibody blocks adherence of neutrophils to anoxic endothelial cells (Yoshida et al., 1992 *Am J Physiol* 262, H1891-8).

Anti-ICAM-1 antibody reduces neurological damage in a rabbit model of cerebral stroke (Bowes et al., 1993 *Exp Neurol* 119, 215-9).

- 20 Anti-ICAM-1 antibody protects against reperfusion injury in a cat model of myocardial ischemia (Ma et al., 1992 *Circulation* 86, 937-46).

- Asthma

Antibody to ICAM-1 partially blocks eosinophil adhesion to endothelial cells and is overexpressed on inflamed airway endothelium and epithelium *in vivo* (Wegner et al., 1990 *Science* 247, 456-9).

- 25 In a primate model of asthma, anti-ICAM-1 antibody blocks airway eosinophilia (Wegner et al., *supra*) and prevents the resurgence of airway inflammation and hyper-responsiveness after dexamethasone treatment (Gundel et al., 1992 *Clin Exp Allergy* 22, 569-75).

- Psoriasis

Surface ICAM-1 and a clipped, soluble version of ICAM-1 is expressed in psoriatic lesions and expression correlates with inflammation (Kellner et al., 1991 *Br J Dermatol* 125, 211-6; Griffiths 1989 *J Am Acad Dermatol* 20, 617-29; Schopf et al., 1993 *Br J Dermatol* 128, 34-7).

- 5 Anti-ICAM antibody blocks keratinocyte antigen presentation to T cells (Nickoloff et al., 1993 *J Immunol* 150, 2148-59).

- Kawasaki disease

Surface ICAM-1 expression correlates with the disease and is reduced by effective immunoglobulin treatment (Leung, et al., 1989 *Lancet* 2, 1298-302).

- 10 Soluble ICAM levels are elevated in Kawasaki disease patients; particularly high levels are observed in patients with coronary artery lesions (Furukawa et al., 1992 *Arthritis Rheum* 35, 672-7; Tsuji, 1992 *Arerugi* 41, 1507-14).

- 15 Circulating LFA-1⁺ T cells are depleted (presumably due to ICAM-1 mediated extravasation) in Kawasaki disease patients (Furukawa et al., 1993 *Scand J Immunol* 37, 377-80).

Example 2: IL-5

- 20 Ribozymes that cleave IL-5 mRNA represent a novel therapeutic approach to inflammatory disorders like asthma. The invention features use of ribozymes to treat chronic asthma, e.g., by inhibiting the synthesis of IL-5 in lymphocytes and preventing the recruitment and activation of eosinophils.

- 25 A number of cytokines besides IL-5 may also be involved in the activation of inflammation in asthmatic patients, including platelet activating factor, IL-1, IL-3, IL-4, GM-CSF, TNF- α , gamma interferon, VCAM, ILAM-1, ELAM-1 and NF- κ B. In addition to these molecules, it is appreciated that any cellular receptors which mediate the activities of the cytokines are also good targets for intervention in inflammatory diseases. These targets include, but are not limited to, the IL-1R and TNF- α R on keratinocytes, epithelial and endothelial cells in airways. Recent data suggest that certain
- 30 neuropeptides may play a role in asthmatic symptoms. These peptides include substance P, neurokinin A and calcitonin-gene-related peptides. These target genes may have more general roles in inflammatory diseases, but are currently assumed to have a role only in asthma.

Ribozymes of this invention block to some extent IL-5 expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of asthma (Clutterbuck et al., 1989 supra; Garssen et al., 1991 Am. Rev. Respir. Dis. 144, 931-938; Larsen et al., 1992 J. Clin. Invest. 89, 747-752; Mauser et al., 1993 supra). Ribozyme cleavage of IL-5 mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

10 The sequence of human and mouse IL-5 mRNA were screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 11, 13, and 14, 15. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most
15 utility. However, mouse targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. (In Table 12, lower case letters indicate positions that are not conserved between the Human and the Mouse IL-5 sequences.)

20 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 12, 14 - 16. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem loop II sequence of
25 hammerhead ribozymes listed in Tables 12 and 14 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 15 and 16 (5'-CACGUUGUG-3') can be altered (substitution, deletion and/or
30 insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables 12, 14 - 16 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

35 By engineering ribozyme motifs we have designed several ribozymes directed against IL-5 mRNA sequences. These ribozymes are synthesized

with modifications that improve their nuclease resistance. The ability of ribozymes to cleave IL-5 target sequences *in vitro* is evaluated.

- The ribozymes will be tested for function *in vivo* by analyzing IL-5 expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA or RNA vectors. IL-5 expression will be monitored by biological assays, ELISA, by indirect immunofluorescence, and/or by FACS analysis. IL-5 mRNA levels will be assessed by Northern analysis, RNase protection or primer extension analysis or quantitative RT-PCR. Ribozymes that block the induction of IL-5 activity and/or IL-5 mRNA by more than 90% will be identified.

Uses

- Interleukin 5 (IL-5), a cytokine produced by CD4⁺ T helper cells and mast cells, was originally termed B cell growth factor II (reviewed by Takatsu et al., 1988 Immunol. Rev. 102, 107). It stimulates proliferation of activated B cells and induces production of IgM and IgA. IL-5 plays a major role in eosinophil function by promoting differentiation (Clutterbuck et al., 1989 Blood 73, 1504-12), vascular adhesion (Walsh et al., 1990 Immunology 71, 258-65) and *in vitro* survival of eosinophils (Lopez et al., 1988 J. Exp. Med. 167, 219-24). This cytokine also enhances histamine release from basophils (Hirai et al., 1990 J. Exp. Med. 172, 1525-8). The following summaries of clinical results support the selection of IL-5 as a primary target for the treatment of asthma:

- Several studies have shown a direct correlation between the number of activated T cells and the number of eosinophils from asthmatic patients vs. normal patients (Oehling et al., 1992 J. Investig. Allergol. Clin. Immunol. 2, 295-9). Patients with either allergic asthma or intrinsic asthma were treated with corticosteroids. The bronchoalveolar lavage was monitored for eosinophils, activated T helper cells and recovery of pulmonary function over a 28 to 30 day period. The number of eosinophils and activated T helper cells decreased progressively with subsequent improvement in pulmonary function compared to intrinsic asthma patients with no corticosteroid treatment.

- Bronchoalveolar lavage cells were screened for production of cytokines using *in situ* hybridization for mRNA. *In situ* hybridization signals

were detected for IL-2, IL-3, IL-4, IL-5 and GM-CSF. Upregulation of mRNA was observed for IL-4, IL-5 and GM-CSF (Robinson et al., 1993 J. Allergy Clin. Immunol. 92, 313-24). Another study showed that upregulation of IL-5 transcripts from allergen challenged vs. saline challenged asthmatic patients (Krishnaswamy et al., 1993 Am. J. Respir. Cell. Mol. Biol. 9, 279-86).

An 18 patient study was performed to determine a mechanism of action for corticosteroid improvement of asthma symptoms. Improvement was monitored by methacholine responsiveness. A correlation was observed between the methacholine responsiveness, a reduction in the number of eosinophils, a reduction in the number of cells expressing IL-4 and IL-5 mRNA and an increase in number of cells expressing interferon-gamma.

Bronchial biopsies from 15 patients were analyzed 24 hours after allergen challenge (Bentley et al., 1993 Am. J. Respir. Cell. Mol. Biol. 8, 35-42). Increased numbers of eosinophils and IL-2 receptor positive cells were found in the biopsies. No differences in the numbers of total leukocytes, T lymphocytes, elastase-positive neutrophils, macrophages or mast cell subtypes were observed. The number of cells expressing IL-5 and GM-CSF mRNA significantly increased.

In another patient study, the eosinophil phenotype was the same for asthmatic patients and normal individuals. However, eosinophils from asthmatic patients had greater leukotriene C4 producing capacity and migration capacity. There were elevated levels of IL-3, IL-5 and GM-CSF in the circulation of asthmatics but not in normal individuals (Bruijnzeel et al., 1992 Schweiz. Med. Wochenschr. 122, 298-301).

Efficacy of antibody to IL-5 was assessed in a guinea pig asthma model. The animals were challenged with ovalbumin and assayed for eosinophilia and the responsiveness to the bronchoconstriction substance P. A 30 mg/kg dose of antibody administered i.p. blocked ovalbumin-induced increased sensitivity to substance P and blocked increases in bronchoalveolar and lung tissue accumulation of eosinophils (Mauser et al., 1993 Am. Rev. Respir. Dis. 148, 1623-7). In a separate study guinea pigs challenged for eight days with ovalbumin were treated with monoclonal antibody to IL-5. Treatment produced a reduction in the

number of eosinophils in bronchoalveolar lavage. No reduction was observed for unchallenged guinea pigs and guinea pigs treated with a control antibody. Antibody treatment completely inhibited the development of hyperreactivity to histamine and arecoline after ovalbumin challenge
5 (van Oosterhout et al., 1993 Am. Rev. Respir. Dis. 147, 548-52)

Results obtained from human clinical analysis and animal studies indicate the role of activated T helper cells, cytokines and eosinophils in asthma. The role of IL-5 in eosinophil development and function makes IL-5 a good candidate for target selection. The antibody studies neutralized
10 IL-5 in the circulation thus preventing eosinophilia. Inhibition of the production of IL-5 will achieve the same goal.

Asthma – a prominent feature of asthma is the infiltration of eosinophils and deposition of toxic eosinophil proteins (e.g. major basic protein, eosinophil-derived neurotoxin) in the lung. A number of T-cell-derived factors like IL-5 are responsible for the activation and maintainance
15 of eosinophils (Kay, 1991 J. Allergy Clin. Immun. 87, 893). Inhibition of IL-5 expression in the lungs can decrease the activation of eosinophils and will help alleviate the symptoms of asthma.

Atopy – is characterized by the developement of type I hypersensitive
20 reactions associated with exposure to certain environmental antigens. One of the common clinical manifestations of atopy is eosinophilia (accumulation of abnormally high levels of eosinophils in the blood). Antibodies against IL-5 have been shown to lower the levels of eosinophils in mice (Cook et al., 1993 in Immunopharmacol. Eosinophils ed. Smith and
25 Cook, pp. 193-216, Academic, London, UK)

Parasitic infection-related eosinophilia– infections with parasites like helminths, can lead to severe eosinophilia (Cook et al., 1993 supra). Animal models for eosinophilia suggest that infection of mice, for example, can lead to blood, peritoneal and/or tissue eosinophilia, all of
30 which seem to be lowered to varying degrees by antibodies directed against IL-5.

Pulmonary infiltration eosinophilia– is characterised by accumulation of high levels of eosinophils in pulmonary parenchyma (Gleich, 1990 J. Allergy Clin. Immunol. 85, 422).

L-Tryptophan-associated eosinophilia-myalgia syndrome (EMS)—

The EMS disease is closely linked to the consumption of L-tryptophan, an essential aminoacid used to treat conditions like insomnia (for review see Varga et al., 1993 J Invest. Dermatol. 100, 97s). Pathologic

5 and histologic studies have demonstrated high levels of eosinophils and mononuclear inflammatory cells in patients with EMS. It appears that IL-5 and transforming growth factor play a significant role in the development of EMS (Varga et al., 1993 supra) by activating eosinophils and other inflammatory cells.

10 Thus, ribozymes of the present invention that cleave IL-5 mRNA and thereby IL-5 activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits IL-5
15 function is described above; available cellular and activity assays are numerous, reproducible, and accurate. Animal models for IL-5 function and for each of the suggested disease targets exist (Cook et al., 1993 supra) and can be used to optimize activity.

Example 3: NF- κ B

Ribozymes that cleave *rel A* mRNA represent a novel therapeutic
20 approach to inflammatory or autoimmune disorders. Inflammatory mediators such as lipopolysaccharide (LPS), interleukin-1 (IL-1) or tumor necrosis factor- α (TNF- α) act on cells by inducing transcription of a number of secondary mediators, including other cytokines and adhesion molecules. In many cases, this gene activation is known to be mediated by
25 the transcriptional regulator, NF- κ B. One subunit of NF- κ B, the *relA* gene product (termed RelA or p65) is implicated specifically in the induction of inflammatory responses. Ribozyme therapy, due to its exquisite specificity, is particularly well-suited to target intracellular factors that contribute to disease pathology. Thus, ribozymes that cleave mRNA encoded by *rel A* or
30 TNF- α may represent novel therapeutics for the treatment of inflammatory and autoimmune disorders.

The nuclear DNA-binding activity, NF- κ B, was first identified as a factor that binds and activates the immunoglobulin κ light chain enhancer in B cells. NF- κ B now is known to activate transcription of a variety of other
35 cellular genes (*e.g.*, cytokines, adhesion proteins, oncogenes and viral

proteins) in response to a variety of stimuli (e.g., phorbol esters, mitogens, cytokines and oxidative stress). In addition, molecular and biochemical characterization of NF- κ B has shown that the activity is due to a homodimer or heterodimer of a family of DNA binding subunits. Each subunit bears a stretch of 300 amino acids that is homologous to the oncogene, *v-rel*. The activity first described as NF- κ B is a heterodimer of p49 or p50 with p65. The p49 and p50 subunits of NF- κ B (encoded by the *nf- κ B2* or *nf- κ B1* genes, respectively) are generated from the precursors NF- κ B1 (p105) or NF- κ B2 (p100). The p65 subunit of NF- κ B (now termed Rel A) is encoded by the *rel A* locus.

The roles of each specific transcription-activating complex now are being elucidated in cells (N.D. Perkins, et al., 1992 Proc. Natl Acad. Sci USA 89, 1529-1533). For instance, the heterodimer of NF- κ B1 and Rel A (p50/p65) activates transcription of the promoter for the adhesion molecule, VCAM-1, while NF- κ B2/RelA heterodimers (p49/p65) actually inhibit transcription (H.B. Shu, et al., *Mol. Cell. Biol.* 13, 6283-6289 (1993)). Conversely, heterodimers of NF- κ B2/RelA (p49/p65) act with Tat-I to activate transcription of the HIV genome, while NF- κ B1/RelA (p50/p65) heterodimers have little effect (J. Liu, N.D. Perkins, R.M. Schmid, G.J. Nabel, *J. Virol.* 1992 66, 3883-3887). Similarly, blocking *rel A* gene expression with antisense oligonucleotides specifically blocks embryonic stem cell adhesion; blocking NF- κ B1 gene expression with antisense oligonucleotides had no effect on cellular adhesion (Narayanan et al., 1993 Mol. Cell. Biol. 13, 3802-3810). Thus, the promiscuous role initially assigned to NF- κ B in transcriptional activation (M.J. Lenardo, D. Baltimore, 1989 Cell 58, 227-229) represents the sum of the activities of the *rel* family of DNA-binding proteins. This conclusion is supported by recent transgenic "knock-out" mice of individual members of the *rel* family. Such "knock-outs" show few developmental defects, suggesting that essential transcriptional activation functions can be performed by more than one member of the *rel* family.

A number of specific inhibitors of NF- κ B function in cells exist, including treatment with phosphorothioate antisense oligonucleotide, treatment with double-stranded NF- κ B binding sites, and over expression of the natural inhibitor MAD-3 (an I κ B family member). These agents have

been used to show that NF- κ B is required for induction of a number of molecules involved in inflammation, as described below.

- NF- κ B is required for phorbol ester-mediated induction of IL-6 (I. Kitajima, et al., Science 258, 1792-5 (1992)) and IL-8 (Kunsch and Rosen, 1993 Mol. Cell. Biol. 13, 6137-46).

•NF- κ B is required for induction of the adhesion molecules ICAM-1 (Eck, et al., 1993 Mol. Cell. Biol. 13, 6530-6536), VCAM-1 (Shu et al., *supra*), and E-selectin (Read, et al., 1994 J. Exp. Med. 179, 503-512) on endothelial cells.

- 10 •NF- κ B is involved in the induction of the integrin subunit, CD18, and other adhesive properties of leukocytes (Eck et al., 1993 *supra*).

The above studies suggest that NF- κ B is integrally involved in the induction of cytokines and adhesion molecules by inflammatory mediators. Two recent papers point to another connection between NF- κ B and inflammation: glucocorticoids may exert their anti-inflammatory effects by inhibiting NF- κ B. The glucocorticoid receptor and p65 both act at NF- κ B binding sites in the ICAM-1 promoter (van de Stolpe, et al., 1994 J. Biol. Chem. 269, 6185-6192). Glucocorticoid receptor inhibits NF- κ B-mediated induction of IL-6 (Ray and Prefontaine, 1994 Proc. Natl Acad. Sci USA 91, 752-756). Conversely, overexpression of p65 inhibits glucocorticoid induction of the mouse mammary tumor virus promoter. Finally, protein cross-linking and co-immunoprecipitation experiments demonstrated direct physical interaction between p65 and the glucocorticoid receptor (*Id.*).

- 25 Ribozymes of this invention block to some extent NF- κ B expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of restenosis, transplant rejection and rheumatoid arthritis. Ribozyme cleavage of *relA* mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

- 30 The sequence of human and mouse *relA* mRNA can be screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 17, 18 and 21-22. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and

ribozymes thereafter designed, the human targetted sequences are of most utility.

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 19 - 22. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity and may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

- 5 By engineering ribozyme motifs we have designed several ribozymes directed against *rel A* mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave *rel A* target sequences *in vitro* is evaluated.

- 10 The ribozymes will be tested for function *in vivo* by analyzing cytokine-induced VCAM-1, ICAM-1, IL-6 and IL-8 expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA and RNA vectors. Cytokine-induced VCAM-1, ICAM-1, IL-6 and IL-8 expression will be monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. *Rel A* mRNA levels will be assessed by Northern analysis, RNAse protection or primer extension analysis or quantitative RT-PCR. Activity of NF- κ B will be monitored by gel-retardation assays. Ribozymes that block the induction of NF- κ B activity and/or *rel A* mRNA by more than 50% will be identified.

- 15 RNA ribozymes and/or genes encoding them will be locally delivered to transplant tissue *ex vivo* in animal models. Expression of the ribozyme will be monitored by its ability to block *ex vivo* induction of VCAM-1, ICAM-1, IL-6 and IL-8 mRNA and protein. The effect of the anti-*rel A* ribozymes on graft rejection will then be assessed. Similarly, ribozymes will be introduced into joints of mice with collagen-induced arthritis or rabbits with *Streptococcal* cell wall-induced arthritis. Liposome delivery, cationic lipid delivery, or adeno-associated virus vector delivery can be used. One dose (or a few infrequent doses) of a stable anti-*rel A* ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate inflammatory and immune responses in these diseases.

Uses

A therapeutic agent that inhibits cytokine gene expression, inhibits adhesion molecule expression, and mimics the anti-inflammatory effects of glucocorticoids (without inducing steroid-responsive genes) is ideal for the treatment of inflammatory and autoimmune disorders. Disease targets for such a drug are numerous. Target indications and the delivery options each entails are summarized below. In all cases, because of the potential immunosuppressive properties of a ribozyme that cleaves *rel A* mRNA, uses are limited to local delivery, acute indications, or *ex vivo* treatment.

10 •Rheumatoid arthritis (RA).

Due to the chronic nature of RA, a gene therapy approach is logical. Delivery of a ribozyme to inflamed joints is mediated by adenovirus, retrovirus, or adeno-associated virus vectors. For instance, the appropriate adenovirus vector can be administered by direct injection into the synovium: high efficiency of gene transfer and expression for several months would be expected (B.J. Roessler, E.D. Allen, J.M. Wilson, J.W. Hartman, B. L. Davidson, J. Clin. Invest. 92, 1085-1092 (1993)). It is unlikely that the course of the disease could be reversed by the transient, local administration of an anti-inflammatory agent. Multiple administrations may be necessary. Retrovirus and adeno-associated virus vectors would lead to permanent gene transfer and expression in the joint. However, permanent expression of a potent anti-inflammatory agent may lead to local immune deficiency.

•Restenosis.

25 Expression of NF- κ B in the vessel wall of pigs causes a narrowing of the luminal space due to excessive deposition of extracellular matrix components. This phenotype is similar to matrix deposition that occurs subsequent to coronary angioplasty. In addition, NF- κ B is required for the expression of the oncogene *c-myc* (F.A. La Rosa, J.W. Pierce, G.E. Sonenshein, Mol. Cell. Biol. 14, 1039-44 (1994)). Thus NF- κ B induces smooth muscle proliferation and the expression of excess matrix components: both processes are thought to contribute to reocclusion of vessels after coronary angioplasty.

•Transplantation.

NF- κ B is required for the induction of adhesion molecules (Eck et al., *supra*, K. O'Brien, et al., J. Clin. Invest. 92, 945-951 (1993)) that function in immune recognition and inflammatory responses. At least two potential modes of treatment are possible. In the first, transplanted organs are treated *ex vivo* with ribozymes or ribozyme expression vectors. Transient inhibition of NF- κ B in the transplanted endothelium may be sufficient to prevent transplant-associated vasculitis and may significantly modulate graft rejection. In the second, donor B cells are treated *ex vivo* with ribozymes or ribozyme expression vectors. Recipients would receive the treatment prior to transplant. Treatment of a recipient with B cells that do not express T cell co-stimulatory molecules (such as ICAM-1, VCAM-1, and/or B7 and B7-2) can induce antigen-specific anergy. Tolerance to the donor's histocompatibility antigens could result; potentially, any donor could be used for any transplantation procedure.

•Asthma.

Granulocyte macrophage colony stimulating factor (GM-CSF) is thought to play a major role in recruitment of eosinophils and other inflammatory cells during the late phase reaction to asthmatic trauma. Again, blocking the local induction of GM-CSF and other inflammatory mediators is likely to reduce the persistent inflammation observed in chronic asthmatics. Aerosol delivery of ribozymes or adenovirus ribozyme expression vectors is a feasible treatment.

•Gene Therapy.

Immune responses limit the efficacy of many gene transfer techniques. Cells transfected with retrovirus vectors have short lifetimes in immune competent individuals. The length of expression of adenovirus vectors in terminally differentiated cells is longer in neonatal or immune-compromised animals. Insertion of a small ribozyme expression cassette that modulates inflammatory and immune responses into existing adenovirus or retrovirus constructs will greatly enhance their potential.

Thus, ribozymes of the present invention that cleave *rel A* mRNA and thereby NF- κ B activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits NF- κ B

function is described above; available cellular and activity assays are number, reproducible, and accurate. Animal models for NF- κ B function (Kitajima, et al., *supra*) and for each of the suggested disease targets exist and can be used to optimize activity.

5 Example 4: TNF- α

Ribozymes that cleave the specific sites in TNF- α mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders.

Tumor necrosis factor- α (TNF- α) is a protein, secreted by activated leukocytes, that is a potent mediator of inflammatory reactions. Injection of
10 TNF- α into experimental animals can simulate the symptoms of systemic and local inflammatory diseases such as septic shock or rheumatoid arthritis.

TNF- α was initially described as a factor secreted by activated macrophages which mediates the destruction of solid tumors in mice (Old,
15 1985 Science 230, 4225-4231). TNF- α subsequently was found to be identical to cachectin, an agent responsible for the weight loss and wasting syndrome associated with tumors and chronic infections (Beutler, et al., 1985 Nature 316, 552-554). The cDNA and the genomic locus for TNF- α have been cloned and found to be related to TNF- β (Shakhov et al., 1990
20 J. Exp. Med. 171, 35-47). Both TNF- α and TNF- β bind to the same receptors and have nearly identical biological activities. The two TNF receptors have been found on most cell types examined (Smith, et al., 1990 Science 248, 1019-1023). TNF- α secretion has been detected from monocytes/macrophages, CD4+ and CD8+ T-cells, B-cells, lymphokine
25 activated killer cells, neutrophils, astrocytes, endothelial cells, smooth muscle cells, as well as various non-hematopoietic tumor cell lines (for a review see Turetskaya et al., 1991 in Tumor Necrosis Factor. Structure, Function, and Mechanism of Action B. B. Aggarwal, J. Vilcek, Eds. Marcel Dekker, Inc., pp. 35-60). TNF- α is regulated transcriptionally and
30 translationally, and requires proteolytic processing at the plasma membrane in order to be secreted (Kriegler et al., 1988 Cell 53, 45-53). Once secreted, the serum half life of TNF- α is approximately 30 minutes. The tight regulation of TNF- α is important due to the extreme toxicity of this cytokine. Increasing evidence indicates that overproduction of TNF- α

during infections can lead to severe systemic toxicity and death (Tracey & Cerami, 1992 Am. J. Trop. Med. Hyg. 47, 2-7).

Antisense RNA and Hammerhead ribozymes have been used in an attempt to lower the expression level of TNF- α by targeting specified cleavage sites [Sioud et al., 1992 J. Mol. Biol. 223; 831; Sioud WO 94/10301; Kisich and co-workers, 1990 abstract (FASEB J. 4, A1860; 1991 slide presentation (J. Leukocyte Biol. sup. 2, 70); December, 1992 poster presentation at Anti-HIV Therapeutics Conference in San Diego, CA; and "Development of anti-TNF- α ribozymes for the control of TNF- α gene expression"- Kisich, Doctoral Dissertation, 1993 University of California, Davis] listing various TNF α targeted ribozymes.

Ribozymes of this invention block to some extent TNF- α expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of septic shock and rheumatoid arthritis. Ribozyme cleavage of TNF- α mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

The sequence of human and mouse TNF- α mRNA can be screened for accessible sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 23, 25, and 27 - 28. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. (In Table 24, lower case letters indicate positions that are not conserved between the human and the mouse TNF- α sequences.)

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 24, 26 - 28. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Tables 24 and 26 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any

sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 27 and 28 (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequences listed in Tables 24, 26 - 28 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables or AAV .

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves TNF- α RNA is inserted into a plasmid DNA vector or an adenovirus DNA viral vector or AAV or alpha virus or retrovirus vectors. Viral vectors have been used to transfer genes to the intact vasculature or to joints of live animals (Willard et al., 1992 Circulation, 86, 1-473.; Nabel et al., 1990 Science, 249, 1285-1288) and both vectors lead to transient gene expression. The adenovirus vector is delivered as recombinant adenoviral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant adenovirus particles are locally administered to the site of treatment, e.g., through the use of an injection catheter, stent or infusion pump or are directly added to cells or tissues *ex vivo*.

In another preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves TNF- α RNA is inserted into a retrovirus vector for sustained expression of ribozyme(s).

By engineering ribozyme motifs we have designed several ribozymes directed against TNF- α mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave TNF- α target sequences *in vitro* is evaluated.

The ribozymes will be tested for function in cells by analyzing bacterial lipopolysaccharide (LPS)-induced TNF- α expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA vectors. TNF- α expression will be monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. TNF- α mRNA levels will be assessed by Northern analysis, RNase protection, primer extension

analysis or quantitative RT-PCR. Ribozymes that block the induction of TNF- α activity and/or TNF- α mRNA by more than 90% will be identified.

RNA ribozymes and/or genes encoding them will be locally delivered to macrophages by intraperitoneal injection. After a period of ribozyme uptake, the peritoneal macrophages are harvested and induced *ex vivo* with LPS. The ribozymes that significantly reduce TNF- α secretion are selected. The TNF- α can also be induced after ribozyme treatment with fixed *Streptococcus* in the peritoneal cavity instead of *ex vivo*. In this fashion the ability of TNF- α ribozymes to block TNF- α secretion in a localized inflammatory response are evaluated. In addition, we will determine if the ribozymes can block an ongoing inflammatory response by delivering the TNF- α ribozymes after induction by the injection of fixed *Streptococcus*.

To examine the effect of anti-TNF- α ribozymes on systemic inflammation, the ribozymes are delivered by intravenous injection. The ability of the ribozymes to inhibit TNF- α secretion and lethal shock caused by systemic LPS administration are assessed. Similarly, TNF- α ribozymes can be introduced into the joints of mice with collagen-induced arthritis. Either free delivery, liposome delivery, cationic lipid delivery, adeno-associated virus vector delivery, adenovirus vector delivery, retrovirus vector delivery or plasmid vector delivery in these animal model experiments can be used to supply ribozymes. One dose (or a few infrequent doses) of a stable anti-TNF- α ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate tissue damage in these inflammatory diseases.

Macrophage isolation.

To produce responsive macrophages 1 ml of sterile fluid thioglycollate broth (Difco, Detroit, MI.) was injected i.p. into 6 week old female C57b1/6NCR mice 3 days before peritoneal lavage. Mice were maintained as specific pathogen free in autoclaved cages in a laminar flow hood and given sterilized water to minimize "spontaneous" activation of macrophages. The resulting peritoneal exudate cells (PEC) were obtained by lavage using Hanks balanced salt solution (HBSS) and were plated at 2.5×10^5 /well in 96 well plates (Costar, Cambridge, MA.) with Eagles minimal essential medium (EMEM) containing 10% heat inactivated fetal

bovine serum. After adhering for 2 hours the wells were washed to remove non-adherent cells. The resulting cultures were 97% macrophages as determined by morphology and staining for non-specific esterase.

Transfection of ribozymes into macrophages:

- 5 The ribozymes were diluted to 2X final concentration, mixed with an equal volume of 11nM lipofectamine (Life Technologies, Gaithersburg, MD.), and vortexed. 100 ml of lipid:ribozyme complex was then added directly to the cells, followed immediately by 10 ml fetal bovine serum. Three hours after ribozyme addition 100 ml of 1 mg/ml bacterial
- 10 lipopolysaccharide (LPS) was added to each well to stimulate TNF production.

Quantitation of TNF- α in mouse macrophages:

- Supernatants were sampled at 0, 2, 4, 8, and 24 hours post LPS stimulation and stored at -70°C. Quantitation of TNF- α was done by a
- 15 specific ELISA. ELISA plates were coated with rabbit anti-mouse TNF- α serum at 1:1000 dilution (Genzyme) followed by blocking with milk proteins and incubation with TNF- α containing supernatants. TNF- α was then detected using a murine TNF- α specific hamster monoclonal antibody (Genzyme). The ELISA was developed with goat anti-hamster IgG coupled
- 20 to alkaline phosphatase.

Assessment of reagent toxicity:

- Following ribozyme/lipid treatment of macrophages and harvesting of supernatants viability of the cells was assessed by incubation of the cells with 5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium
- 25 bromide (MTT). This compound is reduced by the mitochondrial dihydrogenases, the activity of which correlates well with cell viability. After 12 hours the absorbance of reduced MTT is measured at 585 nm.

Uses

- The association between TNF- α and bacterial sepsis, rheumatoid
- 30 arthritis, and autoimmune disease make TNF- α an attractive target for therapeutic intervention [Tracy & Cerami 1992 supra; Williams et al., 1992 Proc. Natl. Acad. Sci. USA 89, 9784-9788; Jacob, 1992 J. Autoimmun. 5 (Supp. A), 133-143].

Septic Shock

Septic shock is a complication of major surgery, bacterial infection, and polytrauma characterized by high fever, increased cardiac output, reduced blood pressure and a neutrophilic infiltrate into the lungs and other major organs. Current treatment options are limited to antibiotics to reduce the bacterial load and non-steroidal anti-inflammatories to reduce fever. Despite these treatments in the best intensive care settings, mortality from septic shock averages 50%, due primarily to multiple organ failure and disseminated vascular coagulation. Septic shock, with an incidence of 200,000 cases per year in the United States, is the major cause of death in intensive care units. In septic shock syndrome, tissue injury or bacterial products initiate massive immune activation, resulting in the secretion of pro-inflammatory cytokines which are not normally detected in the serum, such as TNF- α , interleukin-1 β (IL-1 β), γ -interferon (IFN- γ), interleukin-6 (IL-6), and interleukin-8 (IL-8). Other non-cytokine mediators such as leukotriene b₄, prostaglandin E₂, C3a and C3d also reach high levels (de Boer et al., 1992 Immunopharmacology 24, 135-148).

TNF- α is detected early in the course of septic shock in a large fraction of patients (de Boer et al., 1992 supra). In animal models, injection of TNF- α has been shown to induce shock-like symptoms similar to those induced by LPS injection (Beutler et al., 1985 Science 229, 869-871); in contrast, injection of IL-1 β , IL-6, or IL-8 does not induce shock. Injection of TNF- α also causes an elevation of IL-1 β , IL-6, IL-8, PgE₂, acute phase proteins, and TxA₂ in the serum of experimental animals (de Boer et al., 1992 supra). In animal models the lethal effects of LPS can be blocked by pre-administration of anti-TNF- α antibodies. The cumulative evidence indicates that TNF- α is a key player in the pathogenesis of septic shock, and therefore a good candidate for therapeutic intervention.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the joints leading to bone destruction and loss of joint function. At the cellular level, autoreactive T- lymphocytes and monocytes are typically present, and the synoviocytes often have altered morphology and immunostaining patterns. RA joints have been shown to contain elevated levels of TNF- α , IL-1 α and IL-1 β , IL-6, GM-CSF, and TGF-

β (Abney et al., 1991 Imm. Rev. 119, 105-123), some or all of which may contribute to the pathological course of the disease.

Cells cultured from RA joints spontaneously secrete all of the pro-inflammatory cytokines detected *in vivo*. Addition of antisera against TNF- α to these cultures has been shown to reduce IL-1 α/β production by these cells to undetectable levels (Abney et al., 1991 Supra). Thus, TNF- α may directly induce the production of other cytokines in the RA joint. Addition of the anti-inflammatory cytokine, TGF- β , has no effect on cytokine secretion by RA cultures. Immunocytochemical studies of human RA surgical specimens clearly demonstrate the production of TNF- α , IL-1 α/β , and IL-6 from macrophages near the cartilage/pannus junction when the pannus is invading and overgrowing the cartilage (Chu et al., 1992 Br. J. Rheumatology 31, 653-661). GM-CSF was shown to be produced mainly by vascular endothelium in these samples. Both TNF- α and TGF- β have been shown to be fibroblast growth factors, and may contribute to the accumulation of scar tissue in the RA joint. TNF- α has also been shown to increase osteoclast activity and bone resorption, and may have a role in the bone erosion commonly found in the RA joint (Cooper et al., 1992 Clin. Exp. Immunol. 89, 244-250).

Elimination of TNF- α from the rheumatic joint would be predicted to reduce overall inflammation by reducing induction of MHC class II, IL-1 α/β , IL-6, and GM-CSF, and reducing T-cell activation. Osteoclast activity might also fall, reducing the rate of bone erosion at the joint. Finally, elimination of TNF- α would be expected to reduce accumulation of scar tissue within the joint by removal of a fibroblast growth factor.

Treatment with an anti-TNF- α antibody reduces joint swelling and the histological severity of collagen-induced arthritis in mice (Williams et al., 1992 Proc. Natl. Acad. Sci. USA 89, 9784-9788). In addition, a study of RA patients who have received i.v. infusions of anti-TNF- α monoclonal antibody reports a reduction in the number and severity of inflamed joints after treatment. The benefit of monoclonal antibody treatment in the long term may be limited by the expense and immunogenicity of the antibody.

Psoriasis

Psoriasis is an inflammatory disorder of the skin characterized by keratinocyte hyperproliferation and immune cell infiltrate (Kupper, 1990 J.

Clin. Invest. 86, 1783-1789). It is a fairly common condition, affecting 1.5-2.0% of the population. The disorder ranges in severity from mild, with small flaky patches of skin, to severe, involving inflammation of the entire epidermis. The cellular infiltrate of psoriasis includes T-lymphocytes, neutrophils, macrophages, and dermal dendrocytes. The majority of T-lymphocytes are activated CD4⁺ cells of the T_H-1 phenotype, although some CD8⁺ and CD4⁺/CD8⁺ are also present. B lymphocytes are typically not found in abundance in psoriatic plaques.

Numerous hypotheses have been offered as to the proximal cause of psoriasis including auto-antibodies and auto-reactive T-cells, overproduction of growth factors, and genetic predisposition. Although there is evidence to support the involvement of each of these factors in psoriasis, they are neither mutually exclusive nor are any of them necessary and sufficient for the pathogenesis of psoriasis (Reeves, 1991 Semin. Dermatol. 10, 217).

The role of cytokines in the pathogenesis of psoriasis has been investigated. Among those cytokines found to be abnormally expressed were TGF- α , IL-1 α , IL-1 β , IL-1ra, IL-6, IL-8, IFN- γ , and TNF- α . In addition to abnormal cytokine production, elevated expression of ICAM-1, ELAM-1, and VCAM has been observed (Reeves, 1991 supra). This cytokine profile is similar to that of normal wound healing, with the notable exception that cytokine levels subside upon healing. Keratinocytes themselves have recently been shown to be capable of secreting EGF, TGF- α , IL-6, and TNF- α , which could increase proliferation in an autocrine fashion (Oxholm et al., 1991 APMIS 99, 58-64).

Nickoloff et al., 1993 (J Dermatol Sci. 6, 127-33) have proposed the following model for the initiation and maintenance of the psoriatic plaque:

Tissue damage induces the wound healing response in the skin. Keratinocytes secrete IL-1 α , IL-1 β , IL-6, IL-8, TNF- α . These factors activate the endothelium of dermal capillaries, recruiting PMNs, macrophages, and T-cells into the wound site.

Dermal dendrocytes near the dermal/epidermal junction remain activated when they should return to a quiescent state, and subsequently secrete cytokines including TNF- α , IL-6, and IL-8. Cytokine expression, in

turn, maintains the activated state of the endothelium, allowing extravasation of additional immunocytes, and the activated state of the keratinocytes which secrete TGF- α and IL-8. Keratinocyte IL-8 recruits immunocytes from the dermis into the epidermis. During passage through
5 the dermis, T-cells encounter the activated dermal dendrocytes which efficiently activate the T_H-1 phenotype. The activated T-cells continue to migrate into the epidermis, where they are stimulated by keratinocyte-expressed ICAM-1 and MHC class II. IFN- γ secreted by the T-cells synergizes with the TNF- α from dermal dendrocytes to increase
10 keratinocyte proliferation and the levels of TGF- α , IL-8, and IL-6 production. IFN- γ also feeds back to the dermal dendrocyte, maintaining the activated phenotype and the inflammatory cycle.

Elevated serum titres of IL-6 increases synthesis of acute phase proteins including complement factors by the liver, and antibody production
15 by plasma cells. Increased complement and antibody levels increases the probability of autoimmune reactions.

Maintenance of the psoriatic plaque requires continued expression of all of these processes, but attractive points of therapeutic intervention are TNF- α expression by the dermal dendrocyte to maintain activated
20 endothelium and keratinocytes, and IFN- γ expression by T-cells to maintain activated dermal dendrocytes.

There are 3 million patients in the United States afflicted with psoriasis. The available treatments for psoriasis are corticosteroids. The most widely prescribed are TEMOVATE (clobetasol propionate), LIDEX
25 (fluocinonide), DIPROLENE (betamethasone propionate), PSORCON (diflorasone diacetate) and TRIAMCINOLONE formulated for topical application. The mechanism of action of corticosteroids is multifactorial. This is a palliative therapy because the underlying cause of the disease remains, and upon discontinuation of the treatment the disease returns.
30 Discontinuation of treatment is often prompted by the appearance of adverse effects such as atrophy, telangiectasias and purpura. Corticosteroids are not recommended for prolonged treatments or when treatment of large and/or inflamed areas is required. Alternative treatments include retinoids, such as etretinate, which has been approved for
35 treatment of severe, refractory psoriasis. Alternative retinoid-based treatments are in advanced clinical trials. Retinoids act by converting

keratinocytes to a differentiated state and restoration of normal skin development. Immunosuppressive drugs such as cyclosporine are also in the advanced stages of clinical trials. Due to the nonspecific mechanism of action of corticosteroids, retinoids and immunosuppressives, these treatments exhibit severe side effects and should not be used for extended periods of time unless the condition is life-threatening or disabling. There is a need for a less toxic, effective therapeutic agent in psoriatic patients.

HIV and AIDS

The human immunodeficiency virus (HIV) causes several fundamental changes in the human immune system from the time of infection until the development of full-blown acquired immunodeficiency syndrome (AIDS). These changes include a shift in the ratio of CD4+ to CD8+ T-cells, sustained elevation of IL-4 levels, episodic elevation of TNF- α and TNF- β levels, hypergammaglobulinemia, and lymphoma/leukemia (Rosenberg & Fauci, 1990 Immun. Today 11, 176; Weiss 1993 Science 260, 1273). Many patients experience a unique tumor, Kaposi's sarcoma and/or unusual opportunistic infections (e.g. *Pneumocystis carinii*, cytomegalovirus, herpesviruses, hepatitis viruses, papilloma viruses, and tuberculosis). The immunological dysfunction of individuals with AIDS suggests that some of the pathology may be due to cytokine dysregulation.

Levels of serum TNF- α and IL-6 are often found to be elevated in AIDS patients (Weiss, 1993 supra). In tissue culture, HIV infection of monocytes isolated from healthy individuals stimulates secretion of both TNF- α and IL-6. This response has been reproduced using purified gp120, the viral coat protein responsible for binding to CD-4 (Buonaguro et al., 1992 J. Virol. 66, 7159). It has also been demonstrated that the viral gene regulator, Tat, can directly induce TNF transcription. The ability of HIV to directly stimulate secretion of TNF- α and IL-6 may be an adaptive mechanism of the virus. TNF- α has been shown to upregulate transcription of the LTR of HIV, increasing the number of HIV-specific transcripts in infected cells. IL-6 enhances HIV production, but at a post-transcriptional level, apparently increasing the efficiency with which HIV transcripts are translated into protein. Thus, stimulation of TNF- α secretion by the HIV virus may promote infection of neighboring CD4+ cells both by enhancing virus production from latently infected cells and by driving replication of the virus in newly infected cells.

The role of TNF- α in HIV replication has been well established in tissue culture models of infection (Sher et al., 1992 Immun. Rev. 127, 183), suggesting that the mutual induction of HIV replication and TNF- α replication may create positive feedback *in vivo*. However, evidence for the presence of such positive feedback in infected patients is not abundant. TNF- α levels are found to be elevated in some, but not all patients tested. Children with AIDS who were given zidovudine had reduced levels of TNF- α compared to those not given zidovudine (Cremoni et al., 1993 AIDS 7, 128). This correlation lends support to the hypothesis that reduced viral replication is physiologically linked to TNF- α levels. Furthermore, recently it has been shown that the polyclonal B cell activation associated with HIV infection is due to membrane-bound TNF- α . Thus, levels of secreted TNF- α may not accurately reflect the contribution of this cytokine to AIDS pathogenesis.

Chronic elevation of TNF- α has been shown to result in cachexia (Tracey et al., 1992 Am. J. Trop. Med. Hyg. 47, 2-7), increased autoimmune disease (Jacob, 1992 supra), lethargy, and immune suppression in animal models (Aderka et al., 1992 Isr. J. Med. Sci. 28, 126-130). The cachexia associated with AIDS may be associated with chronically elevated TNF- α frequently observed in AIDS patients. Similarly, TNF- α can stimulate the proliferation of spindle cells isolated from Kaposi's sarcoma lesions of AIDS patients (Barillari et al., 1992 J. Immunol 149, 3727).

A therapeutic agent that inhibits cytokine gene expression, inhibits adhesion molecule expression, and mimics the anti-inflammatory effects of glucocorticoids (without inducing steroid-responsive genes) is ideal for the treatment of inflammatory and autoimmune disorders. Disease targets for such a drug are numerous. Target indications and the delivery options each entails are summarized below. In all cases, because of the potential immunosuppressive properties of a ribozyme that cleaves the specified sites in TNF- α mRNA, uses are limited to local delivery, acute indications, or *ex vivo* treatment.

•Septic shock.

Exogenous delivery of ribozymes to macrophages can be achieved by intraperitoneal or intravenous injections. Ribozymes will be delivered by incorporation into liposomes or by complexing with cationic lipids.

•Rheumatoid arthritis (RA).

- 5 Due to the chronic nature of RA, a gene therapy approach is logical. Delivery of a ribozyme to inflamed joints is mediated by adenovirus, retrovirus, or adeno-associated virus vectors. For instance, the appropriate adenovirus vector can be administered by direct injection into the synovium: high efficiency of gene transfer and expression for several
10 months would be expected (B.J. Roessler, E.D. Allen, J.M. Wilson, J.W. Hartman, B. L. Davidson, J. Clin. Invest. 92, 1085-1092 (1993)). It is unlikely that the course of the disease could be reversed by the transient, local administration of an anti-inflammatory agent. Multiple administrations may be necessary. Retrovirus and adeno-associated virus
15 vectors would lead to permanent gene transfer and expression in the joint. However, permanent expression of a potent anti-inflammatory agent may lead to local immune deficiency.

•Psoriasis

- 20 The psoriatic plaque is a particularly good candidate for ribozyme or vector delivery. The stratum comeum of the plaque is thinned, providing access to the proliferating keratinocytes. T-cells and dermal dendrocytes can be efficiently targeted by trans-epidermal diffusion .

- 25 Organ culture systems for biopsy specimens of psoriatic and normal skin are described in current literature (Nickoloff et al., 1993 Supra). Primary human keratinocytes are easily obtained and will be grown into epidermal sheets in tissue culture. In addition to these tissue culture models, the flaky skin mouse develops psoriatic skin in response to UV light. This model would allow demonstration of animal efficacy for ribozyme treatments of psoriasis.

- 30 •Gene Therapy.

Immune responses limit the efficacy of many gene transfer techniques. Cells transfected with retrovirus vectors have short lifetimes in immune competent individuals. The length of expression of adenovirus

vectors in terminally differentiated cells is longer in neonatal or immune-compromised animals. Insertion of a small ribozyme expression cassette that modulates inflammatory and immune responses into existing adenovirus or retrovirus constructs will greatly enhance their potential.

- 5 Thus, ribozymes of the present invention that cleave TNF- α mRNA and thereby TNF- α activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits TNF- α function is described above; available cellular and activity assays are number, reproducible, and accurate. Animal models for TNF- α function and for each of the suggested disease targets exist and can be used to optimize activity.
- 10

Example 5: p210^{bcr-abl}

- Chronic myelogenous leukemia exhibits a characteristic disease course, presenting initially as a chronic granulocytic hyperplasia, and invariably evolving into an acute leukemia which is caused by the clonal expansion of a cell with a less differentiated phenotype (i.e., the blast crisis stage of the disease). CML is an unstable disease which ultimately progresses to a terminal stage which resembles acute leukemia. This
- 15 lethal disease affects approximately 16,000 patients a year. Chemotherapeutic agents such as hydroxyurea or busulfan can reduce the leukemic burden but do not impact the life expectancy of the patient (e.g. approximately 4 years). Consequently, CML patients are candidates for bone marrow transplantation (BMT) therapy. However, for those patients
- 20 which survive BMT, disease recurrence remains a major obstacle (Apperley et al., 1988 Br. J. Haematol. 69, 239).
- 25

- The Philadelphia (Ph) chromosome which results from the translocation of the *abl* oncogene from chromosome 9 to the *bcr* gene on chromosome 22 is found in greater than 95% of CML patients and in 10-25% of all cases of acute lymphoblastic leukemia [(ALL); Fourth International Workshop on Chromosomes in Leukemia 1982, Cancer Genet. Cytogenet. 11, 316]. In virtually all Ph-positive CMLs and approximately 50% of the Ph-positive ALLs, the leukemic cells express *bcr-abl* fusion mRNAs in which exon 2 (b2-a2 junction) or exon 3 (b3-a2 junction) from the major breakpoint cluster region of the *bcr* gene is spliced
- 30
- 35

- to exon 2 of the *abl* gene. Heisterkamp et al., 1985 Nature 315, 758; Shtivelman et al., 1987, Blood 69, 971). In the remaining cases of Ph-positive ALL, the first exon of the *bcr* gene is spliced to exon 2 of the *abl* gene (Hooberman et al., 1989 Proc. Nat. Acad. Sci. USA 86, 4259;
- 5 Heisterkamp et al., 1988 Nucleic Acids Res. 16, 10069).

- The b3-a2 and b2-a2 fusion mRNAs encode 210 kd bcr-abl fusion proteins which exhibit oncogenic activity (Daley et al., 1990 Science 247, 824; Heisterkamp et al., 1990 Nature 344, 251). The importance of the bcr-abl fusion protein (p210^{bcr-abl}) in the evolution and maintenance of the
- 10 leukemic phenotype in human disease has been demonstrated using antisense oligonucleotide inhibition of p210^{bcr-abl} expression. These inhibitory molecules have been shown to inhibit the in vitro proliferation of leukemic cells in bone marrow from CML patients. Szczylik et al., 1991 Science 253, 562).

- 15 Reddy, U.S. Patent 5,246,921 (hereby incorporated by reference herein) describes use of ribozymes as therapeutic agents for leukemias, such as chronic myelogenous leukemia (CML) by targeting the specific junction region of *bcr-abl* fusion transcripts. It indicates causing cleavage by a ribozyme at or near the breakpoint of such a hybrid chromosome,
- 20 specifically it includes cleavage at the sequence GUX, where X is A, U or G. The one example presented is to cleave the sequence 5' AGC AG AGUU (cleavage site) CAA AAGCCCU-3'.

- Scanlon WO 91/18625, WO 91/18624, and WO 91/18913 and Snyder et al., WO93/03141 and WO94/13793 describe a ribozyme effective
- 25 to cleave oncogenic variants of H-ras RNA. This ribozyme is said to inhibit H-ras expression in response to external stimuli.

- The invention features use of ribozymes to inhibit the development or expression of a transformed phenotype in man and other animals by modulating expression of a gene that contributes to the expression of CML.
- 30 Cleavage of targeted mRNAs expressed in pre-neoplastic and transformed cells elicits inhibition of the transformed state.

The invention can be used to treat cancer or pre-neoplastic conditions. Two preferred administration protocols can be used, either in vivo administration to reduce the tumor burden, or ex vivo treatment to

eradicate transformed cells from tissues such as bone marrow prior to reimplantation.

This invention features an enzymatic RNA molecule (or ribozyme) which cleaves mRNA associated with development or maintenance of CML. The mRNA targets are present in the 425 nucleotides surrounding the fusion sites of the *bcr* and *abl* sequences in the b2-a2 and b3-a2 recombinant mRNAs. Other sequences in the 5' portion of the *bcr* mRNA or the 3' portion of the *abl* mRNA may also be targeted for ribozyme cleavage. Cleavage at any of these sites in the fusion mRNA molecules will result in inhibition of translation of the fusion protein in treated cells.

The invention provides a class of chemical cleaving agents which exhibit a high degree of specificity for the mRNA causative of CML. Such enzymatic RNA molecules can be delivered exogenously or endogenously to afflicted cells. In the preferred hammerhead motif the small size (less than 40 nucleotides, preferably between 32 and 36 nucleotides in length) of the molecule allows the cost of treatment to be reduced.

The smallest ribozyme delivered for any type of treatment reported to date (by Rossi et al., 1992 *supra*) is an *in vitro* transcript having a length of 142 nucleotides. Synthesis of ribozymes greater than 100 nucleotides in length is very difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. Delivery of ribozymes by expression vectors is primarily feasible using only *ex vivo* treatments. This limits the utility of this approach. In this invention, an alternative approach uses smaller ribozyme motifs and exogenous delivery. The simple structure of these molecules also increases the ability of the ribozyme to invade targeted regions of the mRNA structure. Thus, unlike the situation when the hammerhead structure is included within longer transcripts, there are no non-ribozyme flanking sequences to interfere with correct folding of the ribozyme structure, as well as complementary binding of the ribozyme to the mRNA target.

The enzymatic RNA molecules of this invention can be used to treat human CML or precancerous conditions. Affected animals can be treated at the time of cancer detection or in a prophylactic manner. This timing of treatment will reduce the number of affected cells and disable cellular

replication. This is possible because the ribozymes are designed to disable those structures required for successful cellular proliferation.

Ribozymes of this invention block to some extent p210^{bcr-abl} expression and can be used to treat disease or diagnose such disease.

- 5 Ribozymes will be delivered to cells in culture and to tissues in animal models of CML. Ribozyme cleavage of *bcr/abl* mRNA in these systems may prevent or alleviate disease symptoms or conditions.

- 10 The sequence of human *bcr/abl* mRNA can be screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and that contain potential hammerhead or hairpin ribozyme cleavage sites can be identified. These sites are shown in Table 29 (All sequences are 5' to 3' in the tables). The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

- 15 The sequences of the chemically synthesized ribozymes most useful in this study are shown in Table 30. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Table 30 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables 30 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the
25 ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against *bcr-abl* mRNA sequences. These have been synthesized with modifications that improve their nuclease resistance as described above. These ribozymes cleave *bcr-abl* target sequences *in vitro*.

- 30 The ribozymes are tested for function *in vivo* by exogenous delivery to cells expressing *bcr-abl*. Ribozymes are delivered by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA vectors. Expression of *bcr-abl* is monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. Levels of

bcr-abl mRNA are assessed by Northern analysis, RNase protection, by primer extension analysis or by quantitative RT-PCR techniques. Ribozymes that block the induction of p210^{*bcr-abl*} protein and mRNA by more than 20% are identified.

5 Example 6: RSV

This invention relates to the use of ribozymes as inhibitors of respiratory syncytial virus (RSV) production, and in particular, the inhibition of RSV replication.

RSV is a member of the virus family paramyxoviridae and is classified
10 under the genus *Pneumovirus* (for a review see McIntosh and Chanock, 1990 in *Virology* ed. B.N. Fields, pp. 1045, Raven Press Ltd. NY). The infectious virus particle is composed of a nucleocapsid enclosed within an envelope. The nucleocapsid is composed of a linear negative single-stranded non-segmented RNA associated with repeating subunits of
15 capsid proteins to form a compact structure and thereby protect the RNA from nuclease degradation. The entire nucleocapsid is enclosed by the envelope. The size of the virus particle ranges from 150 - 300 nm in diameter. The complete life cycle of RSV takes place in the cytoplasm of infected cells and the nucleocapsid never reaches the nuclear
20 compartment (Hall, 1990 in *Principles and Practice of Infectious Diseases* ed. Mandell et al., Churchill Livingstone, NY).

The RSV genome encodes ten viral proteins essential for viral production. RSV protein products include two structural glycoproteins (G and F) found in the envelope spikes, two matrix proteins [M and M2 (22K)]
25 found in the inner membrane, three proteins localized in the nucleocapsid (N, P and L), one protein that is present on the surface of the infected cell (SH), and two nonstructural proteins [NS1 (1C) and NS2 (1B)] found only in the infected cell. The mRNAs for the 10 RSV proteins have similar 5' and 3' ends. UV-inactivation studies suggest that a single promoter is used
30 with multiple transcription initiation sites (Barik *et al.*, 1992 *J. Virol.* 66, 6813). The order of transcription corresponding to the protein assignment on the genomic RNA is 1C, 1B, N, P, M, SH, G, F, 22K and L genes (Huang *et al.*, 1985 *Virus Res.* 2, 157) and transcript abundance corresponds to the order of gene assignment (for example the 1C and 1B mRNAs are
35 much more abundant than the L mRNA. Synthesis of viral message begins

immediately after RSV infection of cells and reaches a maximum at 14 hours post-infection (McIntosh and Chanock, *supra*).

There are two antigenic subgroups of RSV, A and B, which can circulate simultaneously in the community in varying proportions in different years (McIntosh and Chanock, *supra*). Subgroup A usually predominates. Within the two subgroups there are numerous strains. By the limited sequence analysis available it seems that homology at the nucleotide level is more complete within than between subgroups, although sequence divergence has been noted within subgroups as well. Antigenic determinates result primarily from both surface glycoproteins, F and G. For F, at least half of the neutralization epitopes have been stably maintained over a period of 30 years. For G however, A and B subgroups may be related antigenically by as little as a few percent. On the nucleotide level, however, the majority of the divergence in the coding region of G is found in the sequence for the extracellular domain (Johnson et al., 1987, *Proc. Natl. Acad. Sci. USA* 84, 5625).

Respiratory Syncytial Virus (RSV) is the major cause of lower respiratory tract illness during infancy and childhood (Hall, *supra*) and as such is associated with an estimated 90,000 hospitalizations and 4500 deaths in the United States alone (Update: respiratory syncytial virus activity - United States, 1993, *Mmwr Morb Mortal Wkly Rep*, 42, 971). Infection with RSV generally outranks all other microbial agents leading to both pneumonia and bronchitis. While primarily affecting children under two years of age, immunity is not complete and reinfection of older children and adults, especially hospital care givers (McIntosh and Chanock, *supra*), is not uncommon. Immunocompromised patients are severely affected and RSV infection is a major complication for patients undergoing bone marrow transplantation.

Uneventful RSV respiratory disease resembles a common cold and recovery is in 7 to 12 days. Initial symptoms (rhinorrhea, nasal congestion, slight fever, etc.) are followed in 1 to 3 days by lower respiratory tract signs of infection that include a cough and wheezing. In severe cases, these mild symptoms quickly progress to tachypnea, cyanosis, and listlessness and hospitalization is required. In infants with underlying cardiac or respiratory disease, the progression of symptoms is especially rapid and can lead to respiratory failure by the second or third day of illness. With

modern intensive care however, overall mortality is usually less than 5% of hospitalized patients (McIntosh and Chanock, *supra*).

At present, neither an efficient vaccine nor a specific antiviral agent is available. An immune response to the viral surface glycoproteins can provide resistance to RSV in a number of experimental animals, and a subunit vaccine has been shown to be effective for up to 6 months in children previously hospitalized with an RSV infection (Tristram *et al.*, 1993, J. Infect. Dis. 167, 191). An attenuated bovine RSV vaccine has also been shown to be effective in calves for a similar length of time (Kubota *et al.*, 1992 J. Vet. Med. Sci. 54, 957). Previously however, a formalin-inactivated RSV vaccine was implicated in greater frequency of severe disease in subsequent natural infections with RSV (Connors *et al.*, 1992 J. Virol. 66, 7444).

The current treatment for RSV infection requiring hospitalization is the use of aerosolized ribavirin, a guanosine analog [Antiviral Agents and Viral Diseases of Man, 3rd edition. 1990. (eds. G.J. Galasso, R.J. Whitley, and T.C. Merigan) Raven Press Ltd., NY.]. Ribavirin therapy is associated with a decrease in the severity of the symptoms, improved arterial oxygen and a decrease in the amount of viral shedding at the end of the treatment period. It is not certain, however, whether ribavirin therapy actually shortens the patients' hospital stay or diminishes the need for supportive therapies (McIntosh and Chanock, *supra*). The benefits of ribavirin therapy are especially clear for high risk infants, those with the most serious symptoms or for patients with underlying bronchopulmonary or cardiac disease. Inhibition of the viral polymerase complex is supported as the main mechanism for inhibition of RSV by ribavirin, since viral but not cellular polypeptide synthesis is inhibited by ribavirin in RSV-infected cells (Antiviral Agents and Viral Diseases of Man, 3rd edition. 1990. (eds. G.J. Galasso, R.J. Whitley, and T.C. Merigan) Raven Press Ltd., NY.]. Since ribavirin is at least partially effective against RSV infection when delivered by aerosolization, it can be assumed that the target cells are at or near the epithelial surface. In this regard, RSV antigen had not spread any deeper than the superficial layers of the respiratory epithelium in autopsy studies of fatal pneumonia (McIntosh and Chanock, *supra*).

Jennings *et al.*, WO 94/13688 indicates that targets for specific types of ribozymes include respiratory syncytical virus.

The invention features novel enzymatic RNA molecules, or ribozymes, and methods for their use for inhibiting production of respiratory syncytial virus (RSV). Such ribozymes can be used in a method for treatment of diseases caused by these related viruses in man and other animals. The invention also features cleavage of the genomic RNA and mRNA of these viruses by use of ribozymes. In particular, the ribozyme molecules described are targeted to the *NS1 (1C)*, *NS2 (1B)* and *N* viral genes. These genes are known in the art (for a review see McIntosh and Chanock, 1990 *supra*).

10 Ribozymes that cleave the specified sites in RSV mRNAs represent a novel therapeutic approach to respiratory disorders. Applicant indicates that ribozymes are able to inhibit the activity of RSV and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described
15 that other ribozymes that cleave these sites in RSV mRNAs encoding 1C, 1B and N proteins may be readily designed and are within the invention. Also, those of ordinary skill in the art, will find that it is clear from the examples described that ribozymes cleaving other mRNAs encoded by RSV (*P*, *M*, *SH*, *G*, *F*, *22K* and *L*) and the genomic RNA may be readily
20 designed and are within the invention.

In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables 31, 33, 35, 37 and 38. Examples of such ribozymes are shown in Tables 32, 34, 36-38. Examples of such ribozymes consist essentially of sequences defined in these
25 Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

30 Ribozymes of this invention block to some extent RSV production and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of respiratory disorders. Ribozyme cleavage of RSV encoded mRNAs or the genomic RNA in these systems may alleviate disease symptoms.

While all ten RSV encoded proteins (1C, 1B, N, P, M, SH, 22K, F, G, and L) are essential for viral life cycle and are all potential targets for ribozyme cleavage, certain proteins (mRNAs) are more favorable for ribozyme targeting than the others. For example RSV encoded proteins 1C, 1B, SH and 22K are not found in other members of the family paramyxoviridae and appear to be unique to RSV. In contrast the ectodomain of the G protein and the signal sequence of the F protein show significant sequence divergence at the nucleotide level among various RSV sub-groups (Johnson *et al.*, 1987 *supra*). RSV proteins 1C, 1B and N are highly conserved among various subtypes at both the nucleotide and amino acid levels. Also, 1C, 1B and N are the most abundant of all RSV proteins.

The sequence of human RSV mRNAs encoding 1C, 1B and N proteins are screened for accessible sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 31, 33, 34, 37 and 38 (All sequences are 5' to 3' in the tables.) The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman *et al.*, 1987 J. Am. Chem. Soc., 109, 7845-7854 and in Scaringe *et al.*, 1990 Nucleic Acids Res., 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for G₅ and a U for A₁₄ (numbering from Hertel *et al.*, 1992 Nucleic Acids Res., 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). Hairpin ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant

groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992 *TIBS* 17, 34). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography and are resuspended in water.

5 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 32, 34, 36, 37 and 38. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of
10 hammerhead ribozymes listed in Tables 32 and 34 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in
15 Tables 37 and 38 (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequences listed in Tables 32, 34, 36, 37 and 38 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

20 By engineering ribozyme motifs we have designed several ribozymes directed against RSV encoded mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave target sequences *in vitro* is evaluated.

25 Numerous common cell lines can be infected with RSV for experimental purposes. These include *HeLa*, *Vero* and several primary epithelial cell lines. A cotton rat animal model of experimental human RSV infection is also available, and the bovine RSV is quite homologous to the human viruses. Rapid clinical diagnosis is through the use of kits designed for the immunofluorescence staining of RSV-infected cells or an ELISA
30 assay, both of which are adaptable for experimental study. RSV encoded mRNA levels will be assessed by Northern analysis, RNase protection, primer extension analysis or quantitative RT-PCR. Ribozymes that block the induction of RSV activity and/or 1C, 1B and N protein encoding mRNAs by more than 90% will be identified.

Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described by Draper et al., PCT WO93/23569. The details will not be repeated here, but include altering the length of the ribozyme binding arms or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 Nature 344, 565; Pieken et al., 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International Publication No. WO 91/03162, as well as Jennings et al., WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan, et al., PCT WO94/02595, incorporated by reference herein, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. The RNA/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or stent. Alternative routes of delivery include, but are not limited to, intravenous injection, intramuscular injection, subcutaneous injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan, et al., supra and Draper, et al., supra which have been incorporated by reference herein.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given

pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells

5 (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. U S A, 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet

10 et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. U S A, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. U S A, 90, 6340-4; L'Huillier et al., 1992 EMBO J, 11, 4411-8; Lisiewicz et al., 1993 Proc. Natl. Acad. Sci. U. S. A., 90, 8000-4). The above ribozyme transcription units can be

15 incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral, or alpha virus vectors).

In a preferred embodiment of the invention, a transcription unit

20 expressing a ribozyme that cleaves target RNA is inserted into a plasmid DNA vector, a retrovirus DNA viral vector, an adenovirus DNA viral vector or an adeno-associated virus vector or alpha virus vector. These and other vectors have been used to transfer genes to live animals (for a review see Friedman, 1989 Science 244, 1275-1281; Roemer and Friedman, 1992

25 Eur. J. Biochem. 208, 211-225) and leads to transient or stable gene expression. The vectors are delivered as recombinant viral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant virus particles are locally administered to the site of treatment, e.g., through the

30 use of a catheter, stent or infusion pump.

Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells. The close relationship between ribozyme activity and the structure of the target RNA

35 allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By

using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNA associated with ICAM-1, relA, TNF- α , p210, bcr-abl or RSV related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (i.e., ICAM-1, rel A, TNF α , p210^{bcr-abl} or RSV) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will

decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

II. Chemical Synthesis Of Ribozymes

- 5 There follows the chemical synthesis, deprotection, and purification of RNA, enzymatic RNA or modified RNA molecules in greater than milligram quantities with high biological activity. Applicant has determined that the synthesis of enzymatically active RNA in high yield and quantity is dependent upon certain critical steps used during its preparation.
- 10 Specifically, it is important that the RNA phosphoramidites are coupled efficiently in terms of both yield and time, that correct exocyclic amino protecting groups be used, that the appropriate conditions for the removal of the exocyclic amino protecting groups and the alkylsilyl protecting groups on the 2'-hydroxyl are used, and that the correct work-up and
- 15 purification procedure of the resulting ribozyme be used.

- To obtain a correct synthesis in terms of yield and biological activity of a large RNA molecule (*i.e.*, about 30 to 40 nucleotide bases), the protection of the amino functions of the bases requires either amide or substituted amide protecting groups, which must be, on the one hand, stable enough
- 20 to survive the conditions of synthesis, and on the other hand, removable at the end of the synthesis. These requirements are met by the amide protecting groups shown in Figure 8, in particular, benzoyl for adenosine, isobutyryl or benzoyl for cytidine, and isobutyryl for guanosine, which may be removed at the end of the synthesis by incubating the RNA in NH_3/EtOH
- 25 (ethanolic ammonia) for 20 h at 65 °C. In the case of the phenoxyacetyl type protecting groups shown in Figure 8 on guanosine and adenosine and acetyl protecting groups on cytidine, an incubation in ethanolic ammonia for 4 h at 65 °C is used to obtain complete removal of these protecting groups. Removal of the alkylsilyl 2'-hydroxyl protecting groups
- 30 can be accomplished using a tetrahydrofuran solution of TBAF at room temperature for 8-24 h.

- The most quantitative procedure for recovering the fully deprotected RNA molecule is by either ethanol precipitation, or an anion exchange cartridge desalting, as described in Scaringe *et al. Nucleic Acids Res.*
- 35 1990, 18, 5433-5341. The purification of the long RNA sequences may be

accomplished by a two-step chromatographic procedure in which the molecule is first purified on a reverse phase column with either the trityl group at the 5' position on or off. This purification is accomplished using an acetonitrile gradient with triethylammonium or bicarbonate salts as the aqueous phase. In the case of the trityl on purification, the trityl group may be removed by the addition of an acid and drying of the partially purified RNA molecule. The final purification is carried out on an anion exchange column, using alkali metal perchlorate salt gradients to elute the fully purified RNA molecule as the appropriate metal salts, *e.g.* Na⁺, Li⁺ *etc.* A final de-salting step on a small reverse-phase cartridge completes the purification procedure. Applicant has found that such a procedure not only fails to adversely affect activity of a ribozyme, but may improve its activity to cleave target RNA molecules.

Applicant has also determined that significant (see Tables 39-41) improvements in the yield of desired full length product (FLP) can be obtained by:

1. Using 5-*S*-alkyltetrazole at a delivered or effective concentration of 0.25-0.5 M or 0.15-0.35 M for the activation of the RNA (or analogue) amidite during the coupling step. (By delivered is meant that the actual amount of chemical in the reaction mix is known. This is possible for large scale synthesis since the reaction vessel is of size sufficient to allow such manipulations. The term effective means that available amount of chemical actually provided to the reaction mixture that is able to react with the other reagents present in the mixture. Those skilled in the art will recognize the meaning of these terms from the examples provided herein.) The time for this step is shortened from 10-15 m, *vide supra*, to 5-10 m. Alkyl, as used herein, refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to

7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which have an
5 unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the
10 substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated π electron
15 system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as
20 described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur,
25 and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

30 2. Using 5-S-alkyltetrazole at an effective, or final, concentration of 0.1-0.35 M for the activation of the RNA (or analogue) amidite during the coupling step. The time for this step is shortened from 10-15 m, *vide supra*, to 5-10 m.

3. Using alkylamine (MA, where alkyl is preferably methyl, ethyl,
35 propyl or butyl) or NH₄OH/alkylamine (AMA, with the same preferred alkyl groups as noted for MA) @ 65 °C for 10-15 m to remove the exocyclic

amino protecting groups (vs 4-20 h @ 55-65 °C using NH₄OH/EtOH or NH₃/EtOH, *vide supra*). Other alkylamines, e.g. ethylamine, propylamine, butylamine *etc.* may also be used.

4. Using anhydrous triethylamine•hydrogen fluoride (aHF•TEA)
5 @ 65 °C for 0.5-1.5 h to remove the 2'-hydroxyl alkylsilyl protecting group (vs 8 - 24 h using TBAF, *vide supra* or TEA•3HF for 24 h (Gasparutto *et al. Nucleic Acids Res.* 1992, 20, 5159-5166). Other alkylamine•HF complexes may also be used, e.g. trimethylamine or diisopropylethylamine.

5. The use of anion-exchange resins to purify and/or analyze the
10 fully deprotected RNA. These resins include, but are not limited to, quaternary or tertiary amino derivatized stationary phases such as silica or polystyrene. Specific examples include Dionex-NA100®, Mono-Q®, Poros-Q®.

- Thus, the invention features an improved method for the coupling of
15 RNA phosphoramidites; for the removal of amide or substituted amide protecting groups; and for the removal of 2'-hydroxyl alkylsilyl protecting groups. Such methods enhance the production of RNA or analogs of the type described above (e.g., with substituted 2'-groups), and allow efficient synthesis of large amounts of such RNA. Such RNA may also have
20 enzymatic activity and be purified without loss of that activity. While specific examples are given herein, those in the art will recognize that equivalent chemical reactions can be performed with the alternative chemicals noted above, which can be optimized and selected by routine experimentation.

- In another aspect, the invention features an improved method for the
25 purification or analysis of RNA or enzymatic RNA molecules (e.g. 28-70 nucleotides in length) by passing said RNA or enzymatic RNA molecule over an HPLC, e.g., reverse phase and/or an anion exchange chromatography column. The method of purification improves the catalytic activity of enzymatic RNAs over the gel purification method (see Figure 10).

- 30 Draper *et al.*, PCT WO93/23569, incorporated by reference herein, disclosed reverse phase HPLC purification. The purification of long RNA molecules may be accomplished using anion exchange chromatography, particularly in conjunction with alkali perchlorate salts. This system may be used to purify very long RNA molecules. In particular, it is advantageous to

use a Dionex NucleoPak 100[®] or a Pharmacia Mono Q[®] anion exchange column for the purification of RNA by the anion exchange method. This anion exchange purification may be used following a reverse-phase purification or prior to reverse phase purification. This method results in the formation of a sodium salt of the ribozyme during the chromatography. Replacement of the sodium alkali earth salt by other metal salts, *e.g.*, lithium, magnesium or calcium perchlorate, yields the corresponding salt of the RNA molecule during the purification.

In the case of the 2-step purification procedure, in which the first step is a reverse phase purification followed by an anion exchange step, the reverse phase purification is best accomplished using polymeric, *e.g.* polystyrene based, reverse-phase media, using either a 5'-trityl-on or 5'-trityl-off method. Either molecule may be recovered using this reverse-phase method, and then, once detritylated, the two fractions may be pooled and then submitted to an anion exchange purification step as described above.

The method includes passing the enzymatically active RNA molecule over a reverse phase HPLC column; the enzymatically active RNA molecule is produced in a synthetic chemical method and not by an enzymatic process; and the enzymatic RNA molecule is partially blocked, and the partially blocked enzymatically active RNA molecule is passed over a reverse phase HPLC column to separate it from other RNA molecules.

In more preferred embodiments, the enzymatically active RNA molecule, after passage over the reverse phase HPLC column, is deprotected and passed over a second reverse phase HPLC column (which may be the same as the reverse phase HPLC column), to remove the enzymatic RNA molecule from other components. In addition, the column is a silica or organic polymer-based C4, C8 or C18 column having a porosity of at least 125 Å, preferably 300 Å, and a particle size of at least 2 µm, preferably 5 µm.

Activation

The synthesis of RNA molecules may be accomplished chemically or enzymatically. In the case of chemical synthesis the use of tetrazole as an activator of RNA phosphoramidites is known (Usman *et al.* *J. Am. Chem.*

Soc. 1987, 109, 7845-7854). In this, and subsequent reports, a 0.5 M solution of tetrazole is allowed to react with the RNA phosphoramidite and couple with the polymer bound 5'-hydroxyl group for 10 m. Applicant has determined that using 0.25-0.5 M solutions of 5-S-alkyltetrazoles for only 5 min gives equivalent or better results. The following exemplifies the procedure.

Example 7: Synthesis of RNA and Ribozymes Using 5-S-Alkyltetrazoles as Activating Agent

The method of synthesis used follows the general procedure for RNA synthesis as described in Usman et al., 1987 *supra* and in Scaringe et al., *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The major difference used was the activating agent, 5-S-ethyl or -methyltetrazole @ 0.25 M concentration for 5 min.

All small scale syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 μ mol scale protocol with a reduced 5 min coupling step for alkylsilyl protected RNA and 2.5 m coupling step for 2'-O-methylated RNA. A 6.5-fold excess (162.5 μ L of 0.1 M = 32.5 μ mol) of phosphoramidite and a 40-fold excess of S-ethyl tetrazole (400 μ L of 0.25 M = 100 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% N-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from Applied Biosystems.

All large scale syntheses were conducted on a modified (eight amidite port capacity) 390Z (ABI) synthesizer using a 25 μ mol scale protocol with a 5-15 min coupling step for alkylsilyl protected RNA and 7.5 m coupling step for 2'-O-methylated RNA. A six-fold excess (1.5 mL of 0.1 M = 150 μ mol) of phosphoramidite and a forty-five-fold excess of S-ethyl tetrazole (4.5 mL of

0.25 M = 1125 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 390Z, determined by colorimetric quantitation of the trityl fractions, was 95.0-96.7%. Oligonucleotide synthesis reagents for the 390Z: Detritylation solution was 2% DCA in methylene chloride; capping was performed with 16% *N*-Methylimidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. *S*-Ethyl tetrazole solution (0.25-0.5 M in acetonitrile) was made up from the solid obtained from Applied Biosystems.

Deprotection

The first step of the deprotection of RNA molecules may be accomplished by removal of the exocyclic amino protecting groups with either NH₄OH/EtOH:3/1 (Usman *et al.* *J. Am. Chem. Soc.* 1987, 109, 7845-7854) or NH₃/EtOH (Scaringe *et al.* *Nucleic Acids Res.* 1990, 18, 5433-5341) for ~20 h @ 55-65 °C. Applicant has determined that the use of methylamine or NH₄OH/methylamine for 10-15 min @ 55-65 °C gives equivalent or better results. The following exemplifies the procedure.

Example 8: RNA and Ribozyme Deprotection of Exocyclic Amino Protecting Groups Using Methylamine (MA) or NH₄OH/Methylamine (AMA)

The polymer-bound oligonucleotide, either trityl-on or off, was suspended in a solution of methylamine (MA) or NH₄OH/methylamine (AMA) @ 55-65 °C for 5-15 min to remove the exocyclic amino protecting groups. The polymer-bound oligoribonucleotide was transferred from the synthesis column to a 4 mL glass screw top vial. NH₄OH and aqueous methylamine were pre-mixed in equal volumes. 4 mL of the resulting reagent was added to the vial, equilibrated for 5 m at RT and then heated at 55 or 65 °C for 5-15 min. After cooling to -20 °C, the supernatant was removed from the polymer support. The support was washed with 1.0 mL of EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder. The same procedure was followed for the aqueous methylamine reagent.

Table 40 is a summary of the results obtained using the improvements outlined in this application for base deprotection.

The second step of the deprotection of RNA molecules may be accomplished by removal of the 2'-hydroxyl alkylsilyl protecting group using TBAF for 8-24 h (Usman *et al. J. Am. Chem. Soc.* 1987, 109, 7845-7854). Applicant has determined that the use of anhydrous TEA•HF in *N*-methylpyrrolidine (NMP) for 0.5-1.5 h @ 55-65 °C gives equivalent or better results. The following exemplifies this procedure.

Example 9: RNA and Ribozyme Deprotection of 2'-Hydroxyl Alkylsilyl Protecting Groups Using Anhydrous TEA•HF

To remove the alkylsilyl protecting groups, the ammonia-deprotected oligoribonucleotide was resuspended in 250 µL of 1.4 M anhydrous HF solution (1.5 mL *N*-methylpyrrolidine, 750 µL TEA and 1.0 mL TEA•3HF) and heated to 65 °C for 1.5 h. 9 mL of 50 mM TEAB was added to quench the reaction. The resulting solution was loaded onto a Qiagen 500® anion exchange cartridge (Qiagen Inc.) prewashed with 10 mL of 50 mM TEAB. After washing the cartridge with 10 mL of 50 mM TEAB, the RNA was eluted with 10 mL of 2 M TEAB and dried down to a white powder.

Table 41 is a summary of the results obtained using the improvements outlined in this application for alkylsilyl deprotection.

Example 10: HPLC Purification, Anion Exchange column

For a small scale synthesis, the crude material was diluted to 5 mL with diethylpyrocarbonate treated water. The sample was injected onto either a Pharmacia Mono Q® 16/10 or Dionex NucleoPac® column with 100% buffer A (10 mM NaClO₄). A gradient from 180-210 mM NaClO₄ at a rate of 0.85 mM/void volume for a Pharmacia Mono Q® anion-exchange column or 100-150 mM NaClO₄ at a rate of 1.7 mM/void volume for a Dionex NucleoPac® anion-exchange column was used to elute the RNA. Fractions were analyzed by a HP-1090 HPLC with a Dionex NucleoPac® column. Fractions containing full length product at ≥80% by peak area were pooled.

For a trityl-off large scale synthesis, the crude material was desalted by applying the solution that resulted from quenching of the desilylation reaction to a 53 mL Pharmacia HiLoad 26/10 Q-Sepharose® Fast Flow column. The column was thoroughly washed with 10 mM sodium perchlorate buffer. The oligonucleotide was eluted from the column with

300 mM sodium perchlorate. The eluent was quantitated and an analytical HPLC was run to determine the percent full length material in the synthesis. The eluent was diluted four fold in sterile H₂O to lower the salt concentration and applied to a Pharmacia Mono Q[®] 16/10 column. A
5 gradient from 10-185 mM sodium perchlorate was run over 4 column volumes to elute shorter sequences, the full length product was then eluted in a gradient from 185-214 mM sodium perchlorate in 30 column volumes. The fractions of interest were analyzed on a HP-1090 HPLC with a Dionex NucleoPac[®] column. Fractions containing over 85% full length material
10 were pooled. The pool was applied to a Pharmacia RPC[®] column for desalting.

For a trityl-on large scale synthesis, the crude material was desalted by applying the solution that resulted from quenching of the desilylation reaction to a 53 mL Pharmacia HiLoad 26/10 Q-Sepharose[®] Fast Flow
15 column. The column was thoroughly washed with 20 mM NH₄CO₃H/10% CH₃CN buffer. The oligonucleotide was eluted from the column with 1.5 M NH₄CO₃H/10% acetonitrile. The eluent was quantitated and an analytical HPLC was run to determine the percent full length material present in the synthesis. The oligonucleotide was then applied to a Pharmacia Resource
20 RPC column. A gradient from 20-55% B (20 mM NH₄CO₃H/25% CH₃CN, buffer A = 20 mM NH₄CO₃H/10% CH₃CN) was run over 35 column volumes. The fractions of interest were analyzed on a HP-1090 HPLC with a Dionex NucleoPac[®] column. Fractions containing over 60% full length material were pooled. The pooled fractions were then submitted to manual
25 detritylation with 80% acetic acid, dried down immediately, resuspended in sterile H₂O, dried down and resuspended in H₂O again. This material was analyzed on a HP 1090-HPLC with a Dionex NucleoPac[®] column. The material was purified by anion exchange chromatography as in the trityl-off scheme (*vide supra*).

30 Example 11. Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 μM, 200 nM, 40 nM or 8 nM and the final substrate RNA
35 concentrations were ~ 1 nM. Total reaction volumes were 50 μL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were

initiated by mixing substrate and ribozyme solutions at $t = 0$. Aliquots of 5 μL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each aliquot was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis. Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

Example 12: One pot deprotection of RNA

Applicant has shown that aqueous methyl amine is an efficient reagent to deprotect bases in an RNA molecule. However, in a time consuming step (2-24 hrs), the RNA sample needs to be dried completely prior to the deprotection of the sugar 2'-hydroxyl groups. Additionally, deprotection of RNA synthesized on a large scale (e.g., 100 μmol) becomes challenging since the volume of solid support used is quite large. In an attempt to minimize the time required for deprotection and to simplify the process of deprotection of RNA synthesized on a large scale, applicant describes a one pot deprotection protocol (Fig. 12). According to this protocol, anhydrous methylamine is used in place of aqueous methyl amine. Base deprotection is carried out at 65 °C for 15 min and the reaction is allowed to cool for 10 min. Deprotection of 2'-hydroxyl groups is then carried out in the same container for 90 min in a TEA•3HF reagent. The reaction is quenched with 16 mM TEAB solution.

Referring to Fig. 13, hammerhead ribozyme targeted to site B is synthesized using RNA phosphoramidite chemistry and deprotected using either a two pot or a one pot protocol. Profiles of these ribozymes on an HPLC column are compared. The figure shows that RNAs deprotected by either the one pot or the two pot protocols yield similar full-length product profiles. Applicant has shown that using a one pot deprotection protocol, time required for RNA deprotection can be reduced considerably without compromising the quality or the yield of full length RNA.

Referring to Fig. 14, hammerhead ribozymes targeted to site B (from Fig. 13) are tested for their ability to cleave RNA. As shown in the figure 14, ribozymes that are deprotected using one pot protocol have catalytic activity comparable to ribozymes that are deprotected using a two pot protocol.

Example 12a: Improved protocol for the synthesis of phosphorothioate containing RNA and ribozymes using 5-S-Alkyltetrazoles as Activating Agent

5 The two sulfurizing reagents that have been used to synthesize ribophosphorothioates are tetraethylthiuram disulfide (TETD; Vu and Hirschbein, 1991 *Tetrahedron Letter* 31, 3005), and 3H-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage reagent; Vu and Hirschbein, 1991 *supra*). TETD requires long sulfurization times (600 seconds for DNA and 3600 seconds for RNA). It has recently been shown that for sulfurization of DNA
10 oligonucleotides, Beaucage reagent is more efficient than TETD (Wyrzykiewicz and Ravikumar, 1994 *Bioorganic Med. Chem.* 4, 1519). Beaucage reagent has also been used to synthesize phosphorothioate oligonucleotides containing 2'-deoxy-2'-fluoro modifications wherein the wait time is 10 min (Kawasaki et al., 1992 *J. Med. Chem.*).

15 The method of synthesis used follows the procedure for RNA synthesis as described herein and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The sulfurization step for RNA described in the literature is a 8 second delivery and 10 min wait steps (Beaucage
20 and Iyer, 1991 *Tetrahedron* 49, 6123). These conditions produced about 95% sulfurization as measured by HPLC analysis (Morvan et al., 1990 *Tetrahedron Letter* 31, 7149). This 5% contaminating oxidation could arise from the presence of oxygen dissolved in solvents and/or slow release of traces of iodine adsorbed on the inner surface of delivery lines during
25 previous synthesis.

A major improvement is the use of an activating agent, 5-S-ethyltetrazole or 5-S-methyltetrazole at a concentration of 0.25 M for 5 min. Additionally, for those linkages which are phosphorothioate, the iodine solution is replaced with a 0.05 M solution of 3H-1,2-benzodithiole-3-one
30 1,1-dioxide (Beaucage reagent) in acetonitrile. The delivery time for the sulfurization step is reduced to 5 seconds and the wait time is reduced to 300 seconds.

RNA synthesis is conducted on a 394 (ABI) synthesizer using a modified 2.5 μ mol scale protocol with a reduced 5 min coupling step for
35 alkylsilyl protected RNA and 2.5 min coupling step for 2'-O-methylated RNA. A 6.5-fold excess (162.5 μ L of 0.1 M = 32.5 μ mol) of phosphoramidite

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and a 40-fold excess of *S*-ethyl tetrazole (400 μ L of 0.25 M = 100 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394 synthesizer, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394 synthesizer: detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% *N*-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. *S*-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from Applied Biosystems. Sulfurizing reagent was obtained from Glen Research.

Average sulfurization efficiency (ASE) is determined using the formula: $ASE = (PS/Total)^{1/n-1}$

where, PS = integrated ³¹P NMR values of the P=S diester

Total = integration value of all peaks

n = length of oligo

Referring to tables 42 and 43, effects of varying the delivery and the wait time for sulfurization with Beaucage's reagent is described. These data suggest that 5 second wait time and 300 second delivery time is the condition under which ASE is maximum.

Using the above conditions a 36 mer hammerhead ribozyme is synthesized which is targeted to site C. The ribozyme is synthesized to contain phosphorothioate linkages at four positions towards the 5' end. RNA cleavage activity of this ribozyme is shown in Fig. 16. Activity of the phosphorothioate ribozyme is comparable to the activity of a ribozyme lacking any phosphorothioate linkages.

Example 13: Protocol for the synthesis of 2'-N-phthalimido-nucleoside phosphoramidite

The 2'-amino group of a 2'-deoxy-2'-amino nucleoside is normally protected with *N*-(9-fluorenylmethoxycarbonyl) (Fmoc; Imazawa and Eckstein, 1979 *supra*; Pieken et al., 1991 *Science* 253, 314). This protecting group is not stable in CH₃CN solution or even in dry form during

prolonged storage at -20 °C. These problems need to be overcome in order to achieve large scale synthesis of RNA.

Applicant describes the use of alternative protecting groups for the 2'-amino group of 2'-deoxy-2'-amino nucleoside. Referring to Figure 17,
5 phosphoramidite 17 was synthesized starting from 2'-deoxy-2'-aminonucleoside (12) using transient protection with Markevich reagent (Markiewicz *J. Chem. Res.* 1979, S, 24). An intermediate 13 was obtained in 50% yield, however subsequent introduction of N-phtaloyl (Pht) group by Nefken's method (Nefkens, 1960 *Nature* 185, 306), desilylation (15),
10 dimethoxytrytilation (16) and phosphitylation led to phosphoramidite 17. Since overall yield of this multi-step procedure was low (20%) applicant investigated some alternative approaches, concentrating on selective introduction of N-phtaloyl group without acylation of 5' and 3' hydroxyls.

When 2'-deoxy-2'-amino-nucleoside was reacted with 1.05
15 equivalents of Nefkens reagent in DMF overnight with subsequent treatment with Et₃N (1 hour) only 10-15% of N and 5'(3')-bis-phtaloyl derivatives were formed with the major component being N-Pht-derivative 15. The N,O-bis by-products could be selectively and quantitatively converted to N-Pht derivative 15 by treatment of crude reaction mixture
20 with cat. KCN/MeOH.

A convenient "one-pot" procedure for the synthesis of key intermediate 16 involves selective N-phthaloylation with subsequent dimethoxytrytilation by DMTCl/Et₃N and resulting in the preparation of DMT derivative 16 in 85% overall yield as follows. Standard phosphitylation of
25 16 produced phosphoramidite 17 in 87% yield. One gram of 2'-amino nucleoside, for example 2'-amino uridine (US Biochemicals® part # 77140) was co-evaporated twice from dry dimethyl formamide (Dmf) and dried in vacuo overnight. 50 mls of Aldrich sure-seal Dmf was added to the dry 2'-amino uridine via syringe and the mixture was stirred for 10 minutes
30 to produce a clear solution. 1.0 grams (1.05 eq.) of N-carbethoxyphthalimide (Nefken's reagent, 98% Janssen Chimica) was added and the solution was stirred overnight. Thin layer chromatography (TLC) showed 90% conversion to a faster moving products (10% ETOH in C HCl₃) and 57 µl of TEA (0.1 eq.) was added to effect closure of the
35 phthalimide ring. After 1 hour an additional 855 µl (1.5 eq.) of TEA was added followed by the addition of 1.53 grams (1.1 eq.) of DMT-Cl

(Lancaster Synthesis®, 98%). The reaction mixture was left to stir overnight and quenched with ETOH after TLC showed greater than 90% desired product. Dmf was removed under vacuum and the mixture was washed with sodium bicarbonate solution (5% aq., 500 mls) and extracted with ethyl acetate (2x 200 mls). A 25mm x 300mm flash column (75 grams Merck flash silica) was used for purification. Compound eluted at 80 to 85% ethyl acetate in hexanes (yield: 80% purity: >95% by ¹HNMR). Phosphoramidites were then prepared using standard protocols described above.

- 10 With phosphoramidite 17 in hand applicant synthesized several ribozymes with 2'-deoxy-2'-amino modifications. Analysis of the synthesis demonstrated coupling efficiency in 97-98% range. RNA cleavage activity of ribozymes containing 2'-deoxy-2'-amino-U modifications at U4 and/or U7 positions (see Figure 1), wherein the 2'-amino positions were either
15 protected with Fmoc or Pht, was identical. Additionally, complete deprotection of 2'-deoxy-2'-amino-Uridine was confirmed by base-composition analysis. The coupling efficiency of phosphoramidite 17 was not effected over prolonged storage (1-2 months) at low temperatures.

Protecting 2' Position with a SEM Group

- 20 There follows a method using the 2'-(trimethylsilyl)ethoxymethyl protecting group (SEM) in the synthesis of oligoribonucleotides, and in particular those enzymatic molecules described above. For the synthesis of RNA it is important that the 2'-hydroxyl protecting group be stable throughout the various steps of the synthesis and base deprotection. At the
25 same time, this group should also be readily removed when desired. To that end the *t*-butyldimethylsilyl group has been efficacious (Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* 1990, 18, 5433-5441). However, long exposure times to tetra-*n*-butylammonium fluoride (TBAF) are generally required to fully remove this
30 protecting group from the 2'-hydroxyl. In addition, the bulky alkyl substituents can prove to be a hindrance to coupling thereby necessitating longer coupling times. Finally, it has been shown that the TBDMS group is base labile and is partially deprotected during treatment with ethanolic ammonia (Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* 1990,
35

18, 5433-5441 and Stawinski,J.; Stromberg,R.; Thelin,M.; Westman,E. *Nucleic Acids Res.* 1988, 16, 9285-9298).

The (trimethylsilyl)ethoxymethyl ether (SEM) seems a suitable substitute. This protecting group is stable to base and all but the harshest
5 acidic conditions. Therefore it is stable under the conditions required for oligonucleotide synthesis. It can be readily introduced and the oxygen carbon bond makes it unable to migrate. Finally, the SEM group can be removed with $\text{BF}_3 \cdot \text{OEt}_2$ very quickly.

There follows a method for synthesis of RNA by protecting the 2'-
10 position of a nucleotide during RNA synthesis with a (trimethylsilyl)ethoxymethyl (SEM) group. The method can involve use of standard RNA synthesis conditions as discussed below, or any other equivalent steps. Those in the art are familiar with such steps. The nucleotide used can be any normal nucleotide or may be substituted in
15 various positions by methods well known in the art, e.g., as described by Eckstein *et al.*, *International Publication* No. WO 92/07065, Perrault *et al.*, *Nature* 1990, 344, 565-568, Pieken *et al.*, *Science* 1991, 253, 314-317, Usman,N.; Cedergren,R.J. *Trends in Biochem. Sci.* 1992, 17, 334-339, Usman *et al.*, PCT WO93/15187, and Sproat,B. *European Patent*
20 *Application* 92110298.4.

This invention also features a method for covalently linking a SEM group to the 2'-position of a nucleotide. The method involves contacting a nucleoside with an SEM-containing molecule under SEM bonding
25 conditions. In a preferred embodiment, the conditions are dibutyltin oxide, tetrabutylammonium fluoride and SEM-Cl. Those in the art, however, will recognize that other equivalent conditions can also be used.

In another aspect, the invention features a method for removal of an SEM group from a nucleoside molecule or an oligonucleotide. The method involves contacting the molecule or oligonucleotide with boron trifluoride
30 etherate ($\text{BF}_3 \cdot \text{OEt}_2$) under SEM removing conditions, e.g., in acetonitrile.

Referring to Figure 18, there is shown the method for solid phase synthesis of RNA. A 2',5'-protected nucleotide is contacted with a solid phase bound nucleotide under RNA synthesis conditions to form a dinucleotide. The protecting group (R) at the 2'-position in prior art

methods can be a silyl ether, as shown in the Figure. In the method of the present invention, an SEM group is used in place of the silyl ether. Otherwise RNA synthesis can be performed by standard methodology.

Referring to Figure 19, there is shown the synthesis of 2'-O-SEM
5 protected nucleosides and phosphoramidites. Briefly, a 5'-protected
nucleoside (1) is protected at the 2'- or 3'-position by contacting with a
derivative of SEM under appropriate conditions. Specifically, those
conditions include contacting the nucleoside with dibutyltin oxide and SEM
chloride. The 2 regioisomers are separated by chromatography and the 2'-
10 protected moiety is converted into a phosphoramidite by standard
procedure. The 3'-protected nucleoside is converted into a succinate
derivative suitable for derivatization of a solid support.

Referring to Figure 20, a prior art method for deprotection of RNA using silyl
ethers is shown. This contrasts with the method shown in Figure 21 in
15 which deprotection of RNA containing an SEM group is performed. In step
1, the base protecting groups and cyanoethyl groups are removed by
standard procedure. The SEM group is then removed as shown in the
Figure. The details of the synthesis of phosphoramidites and SEM
protected nucleosides and their use in synthesis of oligonucleotides and
20 subsequent deprotection of

Example 14: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5'-O-Di-
methoxytrityl Uridine (2)

Referring to Figure 19, 5'-O-dimethoxytrityl uridine 1 (1.0 g, 1.83
mmol) in CH₃CN (18 mL) was added dibutyltin oxide (1.0 g, 4.03 mmol)
25 and TBAF (1 M, 2.38 mL, 2.38 mmol). The mixture was stirred for 2 h at RT
(about 20-25°C) at which time (trimethylsilyl)ethoxymethyl chloride (SEM-
Cl) (487 µL, 2.75 mmol) was added. The reaction mixture was stirred
overnight and then filtered and evaporated. Flash chromatography (30%
hexanes in ethyl acetate) yielded 347 mg (28.0%) of 2'-hydroxyl protected
30 nucleoside 2 and 314 mg (25.3%) of 3'-hydroxyl protected nucleoside 3.

Example 15: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl) Uridine (4)

Nucleoside 2 was detritylated following standard methods, as shown
in Figure 19.

Example 16: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5',3'-O-Acetyl Uridine (5)

Nucleoside 4 was acetylated following standard methods, as shown in Figure 19.

5 Example 17: Synthesis of 5',3'-O-Acetyl Uridine (6)

Referring to Figure 19, the fully protected uridine 5 (32 mg, 0.07 mmol) was dissolved in CH₃CN (700 µL) and BF₃•OEt₂ (17.5 µL, 0.14 mmol) was added. The reaction was stirred 15 m and MeOH was added to quench the reaction. Flash chromatography (5% MeOH in CH₂Cl₂) gave
10 20 mg (88%) of SEM deprotected nucleoside 6.

Example 18: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-3'-O-Succinyl-5'-O-Dimethoxytrityl Uridine (2)

Nucleoside 3 was succinylated and coupled to the support following standard procedures, as shown in Figure 19.

15 Example 19: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5'-O-Dimethoxytrityl Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (8)

Nucleoside 3 was phosphitylated following standard methods, as shown in Figure 19.

20 Example 20: Synthesis of RNA Using 2'-O-SEM Protection

Referring to Figure 18, the method of synthesis used follows the general procedure for RNA synthesis as described in Usman, N.; Ogilvie, K.K.; Jiang, M.-Y.; Cadergren, R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucl. Acids Res.*
25 1990, 18, 5433-5441. The phosphoramidite 8 was coupled following standard RNA methods to provide a 10-mer of uridylic acid. Syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 µmol scale protocol with a 10 m coupling step. A thirteen-fold excess (325 µL of 0.1 M = 32.5 µmol) of phosphoramidite and a 80-fold excess of tetrazole
30 (400 µL of 0.5 M = 200 µmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, were 98-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% N-

Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle.

- 5 Referring to Figure 21, the homopolymer was base deprotected with NH₃/EtOH at 65 °C. The solution was decanted and the support was washed twice with a solution of 1:1:1 H₂O:CH₃CN:MeOH. The combined solutions were dried down and then diluted with CH₃CN (1 mL). BF₃•OEt₂ (2.5 μL, 30 μmol) was added to the solution and aliquots were removed at
10 ten time points. The results indicate that after 30 min deprotection is complete, as shown in Figure 22.

III. Vectors Expressing Ribozymes

- There follows a method for expression of a ribozyme in a bacterial or eucaryotic cell, and for production of large amounts of such a ribozyme. In
15 general, the invention features a method for preparing multi-copy cassettes encoding a defined ribozyme structure for production of a ribozyme at a decreased cost. A vector is produced which encodes a plurality of ribozymes which are cleaved at their 3' and 5' ends from an RNA transcript produced from the vector by only one other ribozyme. The system is useful
20 for scaling up production of a ribozyme, which may be either modified or unmodified, *in situ* or *in vitro*. Such vector systems can be used to express a desired ribozyme in a specific cell, or can be used in an *in vitro* system to allow production of large amounts of a desired ribozyme. The vectors of this invention allow a higher yield synthesis of a ribozyme in the form of an
25 RNA transcript which is cleaved *in situ* or *in vitro* before or after transcript isolation.

- Thus, this invention is distinct from the prior art in that a single ribozyme is used to process the 3' and 5' ends of each therapeutic, trans-
acting or desired ribozyme instead of processing only one end, or only one
30 ribozyme. This allows smaller vectors to be derived with multiple trans-acting ribozymes released by only one other ribozyme from the mRNA transcript. Applicant has also provided methods by which the activity of such ribozymes is increased compared to those in the art, by designing ribozyme-encoding vectors and the corresponding transcript such that

folding of the mRNA does not interfere with processing by the releasing ribozyme.

The stability of the ribozyme produced in this method can be enhanced by provision of sequences at the termini of the ribozymes as described by Draper et al., PCT WO 93/23509, hereby incorporated by reference herein.

The method of this invention is advantageous since it provides high yield synthesis of ribozymes by use of low cost transcription-based protocols, compared to existing chemical ribozyme synthesis, and can use isolation techniques currently used to purify chemically synthesized oligonucleotides. Thus, the method allows synthesis of ribozymes in high yield at low cost for analytical, diagnostic, or therapeutic applications.

The method is also useful for synthesis of ribozymes *in vitro* for ribozyme structural studies, enzymatic studies, target RNA accessibility studies, transcription inhibition studies and nuclease protection studies, much is described by Draper et al., PCT WO 93/23509 hereby incorporated by reference herein.

The method can also be used to produce ribozymes *in situ* either to increase the intracellular concentration of a desired therapeutic ribozyme, or to produce a concatameric transcript for subsequent *in vitro* isolation of unit length ribozyme. The desired ribozyme can be used to inhibit gene expression in molecular genetic analyses or in infectious cell systems, and to test the efficacy of a therapeutic molecule or treat afflicted cells.

Thus, in general, the invention features a vector which includes a bacterial, viral or eucaryotic promoter within a plasmid, cosmid, phagemid, virus, viroid, virusoid or phage vector. Other vectors are equally suitable and include double-stranded, or partially double-stranded DNA, formed by an amplification method such as the polymerase chain reaction, or double-stranded, partially double-stranded or single-stranded RNA, formed by site-directed homologous recombination into viral or viroid RNA genomes. Such vectors need not be circular. Transcriptionally linked to the promoter region is a first ribozyme-encoding region, and nucleotide sequences encoding a ribozyme cleavage sequence which is placed on either side of a region encoding a therapeutic or otherwise desired second ribozyme.

Suitable restriction endonuclease sites can be provided to ease construction of this vector in DNA vectors or in requisite DNA vectors of an RNA expression system. The desired second ribozyme may be any desired type of ribozyme, such as a hammerhead, hairpin, hepatitis delta virus (HDV) or other catalytic center, and can include group I and group II introns, as discussed above. The first ribozyme is chosen to cleave the encoded cleavage sequence, and may also be any desired ribozyme, for example, a *Tetrahymena* derived ribozyme, which may, for example, include an imbedded restriction endonuclease site in the center of a self-recognition sequence to aid in vector construction. This endonuclease site is useful for construction of the vector, and subsequent analysis of the vector.

When the promoter of such a vector is activated an RNA transcript is produced which includes the first and second ribozyme sequences. The first ribozyme sequence is able to act, under appropriate conditions, to cause cleavage at the cleavage sites to release the second ribozyme sequences. These second ribozyme sequences can then act at their target RNA sites, or can be isolated for later use or analysis.

Thus, in one aspect the invention features a vector which includes a first nucleic acid sequence (encoding a first ribozyme having intramolecular cleaving activity), and a second nucleic acid sequence (encoding a second ribozyme having intermolecular cleaving enzymatic activity) flanked by nucleic acid sequences encoding RNA which is cleaved by the first ribozyme to release the second ribozyme from the RNA transcript encoded by the vector. The second ribozyme may be flanked by the first ribozyme either on the 5' side or 3' side. If desired, the first ribozyme may be encoded on a separate vector and may have intermolecular cleaving activity.

As discussed above, the first ribozyme can be chosen to be any self-cleaving ribozyme, and the second ribozyme may be chosen to be any desired ribozyme. The flanking sequences are chosen to include sequences recognized by the first ribozyme. When the vector is caused to express RNA from these nucleic acid sequences, that RNA has the ability under appropriate conditions to cleave each of the flanking regions and thereby release one or more copies of the second ribozyme. If desired, several different second ribozymes can be produced by the same vector, or

several different vectors can be placed in the same vessel or cell to produce different ribozymes.

In preferred embodiments, the vector includes a plurality of the nucleic acid sequences encoding the second ribozyme, each flanked by nucleic acid sequences recognized by the first ribozyme. Most preferably, such a plurality includes at least six to nine or even between 60 - 100 nucleic acid sequences. In other preferred embodiments, the vector includes a promoter which regulates expression of the nucleic acid encoding the ribozymes from the vector; and the vector is chosen from a plasmid, cosmid, phagmid, virus, viroid or phage. In a most preferred embodiment, the plurality of nucleic acid sequences are identical and are arranged in sequential order such that each has an identical end nearest to the promoter. If desired, a poly(A) sequence adjacent to the sequence encoding the first or second ribozyme may be provided to increase stability of the RNA produced by the vector; and a restriction endonuclease site adjacent to the nucleic acid encoding the first ribozyme is provided to allow insertion of nucleic acid encoding the second ribozyme during construction of the vector.

In a second aspect, the invention features a method for formation of a ribozyme expression vector by providing a vector including nucleic acid encoding a first ribozyme, as discussed above, and providing a single-stranded DNA encoding a second ribozyme, as discussed above. The single-stranded DNA is then allowed to anneal to form a partial duplex DNA which can be filled in by a treatment with an appropriate enzyme, such as a DNA polymerase in the presence of dNTPs, to form a duplex DNA which can then be ligated to the vector. Large vectors resulting from this method can then be selected to insure that a high copy number of the single-stranded DNA encoding the second ribozyme is incorporated into the vector.

In a further aspect, the invention features a method for production of ribozymes by providing a vector as described above, expressing RNA from that vector, and allowing cleavage by the first ribozyme to release the second ribozyme.

In preferred embodiments, three different ribozyme motifs are used as cis-cleaving ribozymes. The hammerhead, hairpin, and hepatitis delta

virus (HDV) ribozyme motifs consist of small, well-defined sequences that rapidly self-cleave *in vitro* (Symons, 1992 Annu. Rev. Biochem. 61, 641). While structural and functional differences exist among the three ribozyme motifs, they self-process efficiently *in vivo*. All three ribozyme motifs self-process to 87-95% completion in the absence of 3' flanking sequences. *In vitro*, the self-processing constructs described in this invention are significantly more active than those reported by Taira et al., 1990 supra; and Altschuler et al., 1992 Gene 122, 85. The present invention enables the use of cis-cleaving ribozymes to efficiently truncate RNA molecules at specific sites *in vivo* by ensuring lack of secondary structure which prevents processing.

Isolation of Therapeutic Ribozyme

The preferred method of isolating therapeutic ribozyme is by a chromatographic technique. The HPLC purification methods and reverse HPLC purification methods described by Draper et al., PCT WO 93/23509, hereby incorporated by reference herein, can be used. Alternatively, the attachment of complementary oligonucleotides to cellulose or other chromatography columns allows isolation of the therapeutic second ribozyme, for example, by hybridization to the region between the flanking arms and the enzymatic RNA. This hybridization will select against the short flanking sequences without the desired enzymatic RNA, and against the releasing first ribozyme. The hybridization can be accomplished in the presence of a chaotropic agent to prevent nuclease degradation. The oligonucleotides on the matrix can be modified to minimize nuclease activity, for example, by provision of 2'-O-methyl RNA oligonucleotides. Such modifications of the oligonucleotide attached to the column matrix will allow the multiple use of the column with minimal oligo degradation. Many such modifications are known in the art, but a chemically stable non-reducible modification is preferred. For example, phosphorothioate modifications can also be used.

The expressed ribozyme RNA can be isolated from bacterial or eucaryotic cells by routine procedures such as lysis followed by guanidine isothiocyanate isolation.

The current known self-cleaving site of *Tetrahymena* can be used in an alternative vector of this invention. If desired, the full-length

Tetrahymena sequence may be used, or a shorter sequence may be used. It is preferred that, in order to decrease the superfluous sequences in the self-cleaving site at the 5' cleavage end, the hairpin normally present in the *Tetrahymena* ribozyme should contain the therapeutic second ribozyme 3' sequence and its complement. That is, the first releasing ribozyme-encoding DNA is provided in two portions, separated by DNA encoding the desired second ribozyme. For example, if the therapeutic second ribozyme recognition sequence is CGGACGA/CGAGGA, then CGAGGA is provided in the self-cleaving site loop such that it is in a stem structure recognized by the *Tetrahymena* ribozyme. The loop of the stem may include a restriction endonuclease site into which the desired second ribozyme-encoding DNA is placed.

If desired, the vector may be used in a therapeutic protocol by use of the systems described by Lechner, PCT WO 92/13070, hereby incorporated by reference herein, to allow a timed expression of the therapeutic second ribozyme, as well as an appropriate shut off of cell or gene function. Thus, the vector will include a promoter which appropriately expresses enzymatically active RNA only in the presence of an RNA or another molecule which indicates the presence of an undesired organism or state. Such enzymatically active RNA will then kill or harm the cell in which it exists, as described by Lechner, *id.*, or act to cause reduced expression of a desired protein product.

A number of suitable RNA vectors may also be used in this invention. The vectors include plant viroids, plant viruses which contain single or double-stranded RNA genomes and animal viruses which contain RNA genomes, such as the picornaviruses, myxoviruses, paramyxoviruses, hepatitis A virus, reovirus and retroviruses. In many instances cited, use of these viral vectors also results in tissue specific delivery of the ribozymes.

Example 21: Design of self-processing cassettes

In a preferred embodiment, applicant compared the *in vitro* and *in vivo* cis-cleaving activity of three different ribozyme motifs—the hammerhead, the hairpin and the hepatitis delta virus ribozyme—in order to assess their potential to process the ends of transcripts *in vivo*. To make a direct comparison among the three, however, it is important to design the ribozyme-containing transcripts to be as similar as possible. To this end,

all the ribozyme cassettes contained the same trans-acting hammerhead ribozyme followed immediately by one of the three cis-acting ribozymes (Figure 23-25). For simplicity, applicant refers to each cassette by an abbreviation that indicates the downstream cis-cleaving ribozyme only.

- 5 Thus HH refers to the cis-cleaving cassette containing a hammerhead ribozyme, while HP and HDV refer to the cassettes containing hairpin and hepatitis delta virus cis-cleaving ribozymes, respectively. The general design of the ribozyme cassettes, as well as specific differences among the cassettes, are outlined below.

- 10 A sequence predicted to form a stable stem-loop structure is included at the 5' end of all the transcripts. The hairpin stem contains the T7 RNA polymerase initiation sequence (Milligan & Uhlenbeck, 1989 Methods Enzymol. 180, 51) and its complement, separated by a stable tetra-loop (Antao et al., 1991 Nucleic Acids Res. 19, 5901). By incorporating the T7
15 initiation sequence into a stem-loop structure, applicant hoped to avoid nonproductive base pairing interactions with either the trans-acting ribozyme or with the cis-acting ribozyme. The presence of a hairpin at the end of a transcript may also contribute to the stability of the transcript *in vivo*. These are non-limiting examples. Those in the art will recognize that
20 other embodiments can be readily generated using a variety of promoters, initiator sequences and stem-loop structure combinations generally known in the art.

- The trans-acting ribozyme used in this study is targeted to a site B (5'...CUGGAGUC↓GACCUUC...3'). The 5' binding arm of the ribozyme, 5'-
25 GAAGGUC-3', and the core of the ribozyme, 5'-CUGAUGAGGCCGAAAGGCCGAA-3', remain constant in all cases. In addition, all transcripts also contain a single nucleotide between the 5' stem-loop and the first nucleotide of the ribozyme. The linker nucleotide was required to obtain the same activity *in vitro* that was measured with an
30 identical ribozyme lacking the 5' hairpin. Because the three cis-cleaving ribozymes have different requirements at the site of cleavage, slight differences were unavoidable at the 3' end of the processed transcript. The junction between the trans- and cis-acting ribozyme is, however, designed so that there is minimal extraneous sequence left at the 3' end of the trans-
35 cleaving ribozyme once cis-cleavage occurs. The only differences between the constructs lie in the 3' binding arm of the ribozyme, where

either 6 or 7 nucleotides, 5'-ACUCCA(+/-G)-3', complementary to the target sequence are present and where, after processing, two to five extra nucleotides remain.

The cis-cleaving hammerhead ribozyme used in the HH cassette is based on the design of Grosshans and Cech, 1991 supra. As shown in Figure 23, the 3' binding arm of the trans-acting ribozyme is included in the required base-pairing interactions of the cis-cleaving ribozyme to form stem I. Two extra nucleotides, UC, were included at the end of the 3' binding arm to form the self-processing hammerhead ribozyme site (Ruffner et al., 1990 supra) which remain on the 3' end of the trans-acting ribozyme following self-processing.

The hairpin ribozyme portion of the HP self-processing construct is based on the minimal wild-type sequence (Hampel & Tritz, 1989 supra). A tetra-loop at the end of helix 1 (3' side of the cleavage site) serves to link the two portions and thus allows a minimal five nucleotides to remain at the end of the released trans-acting ribozyme following self-processing. Two variants of HP were designed: HP(GU) and HP(GC). The HP(GU) was constructed with a G-U wobble base pair in helix 2 (A52G substitution; Figure 24). This slight destabilization of helix 2 was intended to improve self-processing activity by promoting product release and preventing the reverse reaction (Berzal-Herranz et al., 1992 Genes & Dev. 6, 129; Chowrira et al., 1993 Biochemistry 32, 1088). The HP(GC) cassette was constructed as a control for strong base-pairing interactions in helix 2 (U77C and A52G substitution; Figure 24). Another modification to discourage the reverse ligation reaction of the hairpin ribozyme was to shorten helix 1 (Figure 24) by one base pair relative to the wild-type sequence (Chowrira & Burke, 1991 Biochemistry 30, 8518).

The HDV ribozyme self-processes efficiently when the nucleotide 5' to the cleavage site is a pyrimidine, and somewhat less so when adenosine is in that position. No other sequence requirements have been identified upstream of the cleavage site, however, we have observed some decrease in activity when a stem-loop structure was present within 2 nt of the cleavage site. The HDV self-processing construct (Fig 25) was designed to generate the trans-acting hammerhead ribozyme with only two additional nucleotides at its 3' end after self-processing. The HDV sequence used here is based on the anti-genomic sequence (Perrota & Been, 1992 supra)

but includes the modifications of Been et al., 1992 (Biochemistry 31, 11843) in which cis-cleavage activity of the ribozyme was improved by the substitution of a shortened helix 4 for a wild-type stem-loop (Figure 25).

To prepare DNA inserts that encode self-processing ribozyme cassettes, partially overlapping top- and bottom-strand oligonucleotides (60-90 nucleotides) were designed to include sequences for the T7 promoter, the trans-acting ribozyme, the cis-cleaving ribozyme and appropriate restriction sites for use in cloning (see Fig. 26). The single-strand portions of annealed oligonucleotides were converted to double-strands using Sequenase® (U.S. Biochemicals). Insert DNA was ligated into *EcoRI*/*HindIII*-digested puc18 and transformed into *E. coli* strain DH5 α using standard protocols (Maniatis et al., 1982 in Molecular Cloning Cold Spring Harbor Press). The identity of positive clones was confirmed by sequencing small-scale plasmid preparations.

Larger scale preparations of plasmid DNA for use as *in vitro* transcription templates and in transactions were prepared using the protocol and columns from QIAGEN Inc. (Studio City, CA) except that an additional ethanol precipitation was included as the final step.

Example 22: RNA Processing *in vitro*

Transcription reactions containing linear plasmid templates were carried out essentially as described (Milligan & Uhlenbeck, 1989 Supra; Chowrira & Burke, 1991 Supra). In order to prepare 5' end-labeled transcripts, standard transcription reactions were carried out in the presence of 10-20 μ Ci [γ -³²P]GTP, 200 μ M each NTP and 0.5 to 1 μ g of linearized plasmid template. The concentration of MgCl₂ was maintained at 10 mM above the total nucleotide concentration.

To compare the ability of the different ribozyme cassettes to self-process *in vitro*, each construct was transcribed and allowed to undergo self-processing under identical conditions at 37°C. For these comparisons, equal amounts of linearized DNA templates bearing the various ribozyme cassettes were transcribed in the presence of [γ -³²P]GTP to generate 5' end-labeled transcripts. In this manner only the full-length, unprocessed transcripts and the released trans-ribozymes are visualized by autoradiography. In all reactions, Mg²⁺ was included at 10 mM above the nucleotide concentration so that cleavage by all the ribozyme cassettes

would be supported. Transcription templates were linearized at several positions by digestion with different restriction enzymes so that self-processing in the presence of increasing lengths of downstream sequence could be compared (see Fig. 26). The resulting transcripts have either 4-5
5 non-ribozyme nucleotides at the 3' end (*HindIII*-digested template), 220 nucleotides (*NdeI* digested templates) or 454 nucleotides of downstream sequence (*RcaI* digested template).

As shown in Figure 27, all four ribozyme cassettes are capable of self-processing and yield RNA products of expected sizes. Two nucleotides
10 essential for hammerhead ribozyme activity (Ruffner et al., 1990 *supra*) have been changed in the HH(mutant) core sequence (see Figure 23) and so this transcript is unable to undergo self-processing (Fig. 27). This is evidenced by the lack of a released 5' RNA in the HH(mutant), although the full-length RNAs are present. Comparison of the amounts of released
15 trans-ribozyme (Fig. 27) indicate that there are differences in the ability of these ribozymes to self-process *in vitro*, especially with respect to the presence of downstream sequence. For the two HP constructs, it is clear that HP(GC) is more efficient than the HP(GU) ribozyme, both in the presence and in the absence of extra downstream sequence. In addition,
20 the activity of HP(GU) falls off more dramatically when downstream sequence is present. The stronger G:C base pair likely contributes to the HP(GC) construct's ability to fold correctly (and/or more quickly) into the productive structure, even when as much as 216 extra nucleotides are present downstream. The HH ribozyme construct is also quite efficient at
25 self-processing, and slightly better than the HP(GU) construct even when downstream sequence is present.

Of the three ribozyme motifs, the presence of extra downstream sequence seems to most affect the efficiency of HDV. When no extra sequence is present downstream, HDV is quite efficient and self-processes
30 to approximately the same level as the HH and HP(GC) cassettes. However, when extra downstream sequence is present, the self-processing activity seems to decrease almost as dramatically as is seen with the (sub-optimal) HP(GU) cassette.

Example 23: Kinetics of self-processing reaction

*Hind*III-digested template (250 ng) was used in a standard transcription reaction mixture containing: 50 mM Tris-HCl pH 8.3; 1 mM ATP, GTP and UTP; 50 μ M CTP; 40 μ Ci [α - 32 P]CTP; 12 mM MgCl₂; 10 mM DTT. The transcription/self-processing reaction was initiated by the addition of T7 RNA polymerase (15 U/ μ l). Aliquots of 5 μ l were taken at regular time intervals and the reaction was stopped by adding an equal volume of 2x formamide loading buffer (95% formamide, 15 mM EDTA, & dyes) and freezing on dry ice. The samples were resolved on a 10% polyacrylamide sequencing gel and results were quantitated by PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Ribozyme self-cleavage rates were determined from non-linear, least-squares fits (KaleidaGraph, Synergy Software, Reading, PA) of the data to the equation:

$$(\text{Fraction Uncleaved Transcript}) = \frac{1}{kt} (1 - e^{-kt})$$

where *t* represents time and *k* represents the unimolecular rate constant for cleavage (Long & Uhlenbeck, 1994 Proc. Natl. Acad. Sci. USA 91, 6977).

Linear templates were prepared by digesting the plasmids with *Hind*III so that transcripts will contain only four to five vector-derived nucleotides at the 3' end (see Figure 23-25). By comparison of the unimolecular rate constant (*k*) determined for each construct, it is clear that HH is the most efficient at self-processing (Table 44). The HH transcript self-processes 2-fold faster than HDV and 3-fold faster than HP(GC) transcripts. Although the HP(GU) RNA undergoes self-processing, it is at least 6-fold slower than the HP(GC) construct. This is consistent with previous observations that the stability of helix 2 is essential for self-processing and trans-cleavage activity of the hairpin ribozyme (Hampel et al., 1990 supra; Chowrira & Burke, 1991 supra). The rate of HH self-cleavage during transcription measured here (1.2 min⁻¹) is similar to the rate measured by Long and Uhlenbeck 1994 supra using a HH that has a different stem I and stem III. Self-processing rates during transcription for HP and HDV have not been previously reported. However, self-processing of the HDV ribozyme—as measured here during transcription—is significantly slower than when tested after isolation from a denaturing gel (Been et al., 1992 supra). This decrease likely reflects the difference in protocol as well as the presence of 5' flanking sequence in the HDV construct used here.

Example 24: Effect of downstream sequences on trans-cleavage *in vitro*

Transcripts containing the trans ribozyme with or without 3' flanking sequences were assayed for their ability to cleave their target *in trans*. To this end, transcripts from three templates were resolved on a preparative gel and bands corresponding both to processed trans-acting ribozymes from the HH transcription reaction, and to full-length HH(mutant) and Δ HDV transcripts were isolated. In all three transcripts the trans-acting ribozyme portion is identical—with the exception of sequences at their 3' ends. The HH trans-acting ribozyme contains only an additional UC at its 3' end, while HH(mutant) and Δ HDV have 52 and 37 nucleotides, respectively, at their 3' ends. A 622 nucleotide, internally-labeled target RNA was incubated, under ribozyme excess conditions, along with the three ribozyme transcripts in a standard reaction buffer.

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 622 nt region (containing hammerhead site P) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed in a standard transcription buffer in the presence of [α - 32 P]CTP (Chowrira & Burke, 1991 *supra*). The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (1 μ M) and internally labeled 622 nt substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager® (Molecular Dynamics, Sunnyvale, CA).

The HH trans-acting ribozyme cleaves the target RNA approximately 10-fold faster than the Δ HDV transcript and greater than 20-fold faster than

the HH(mutant) transcript (Figure 28). The additional nucleotides at the end of HH(mutant) form 7 base-pairs with the 3' target-binding arm of the trans-acting ribozyme (Figure 23). This interaction must be disrupted (at a cost of 6 kcal/mole) to make the trans-acting ribozyme available for binding the target sequence. In contrast, the additional nucleotides at the end of Δ HDV were not designed to form any strong, alternative base-pairing with the trans-ribozyme. Nevertheless, the Δ HDV sequences are predicted to form multiple structures involving the 3' target-binding arm of the trans ribozyme that have stabilities ranging from 1-2 kcal/mole. Thus, the observed reductions in activity for the Δ HDV and HH(mutant) constructs are consistent with the predicted folded structures, and it reinforces the view that the flanking sequences can decrease the catalytic efficiency of a ribozyme through nonproductive interactions with either the ribozyme or the substrate or both.

15 Example 25: RNA self-processing *in vivo*

Since three of the constructs (HH, HDV and HP(GC)) self-process efficiently in solution, the affect of the mammalian cellular milieu on ribozyme self-processing was next explored by applicant. A transient expression system was employed to investigate ribozyme activity *in vivo*. A mouse cell line (OST7-1) that constitutively expresses T7 RNA polymerase in the cytoplasm was chosen for this study (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. USA 87, 6743). In these cells plasmids containing a ribozyme cassette downstream of the T7 promoter will be transcribed efficiently in the cytoplasm (Elroy-Stein & Moss, 1990 supra).

25 Monolayers of a mouse L9 fibroblast cell line (OST7-1; Elroy-Stein and Moss, 1990 supra) were grown in 6-well plates with $\sim 5 \times 10^5$ cells/well. Cells were transfected with circular plasmids (5 μ g/well) using the calcium phosphate-DNA precipitation method (Maniatis et al., 1982 supra). Cells were lysed (4 hours post-transfection) by the addition of standard lysis buffer (200 μ l/well) containing 4M guanadinium isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarkosyl (Chomczynski and Sacchi, 1987 Anal. Biochem. 162, 156), and 50 mM EDTA pH 8.0. The lysate was extracted once with water-saturated phenol followed by one extraction with chloroform:isoamyl alcohol (25:1). Total cellular RNA was precipitated with an equal volume of isopropanol. The RNA pellet was resuspended in 0.2

M ammonium acetate and reprecipitated with ethanol. The pellet was then washed with 70% ethanol and resuspended in DEPC-treated water.

Purified cellular RNA (3 µg/reaction) was first denatured in the presence of a 5' end-labeled DNA primer (100 pmol) by heating to 90°C for 2 min. in the absence of Mg²⁺, and then snap-cooling on ice for at least 15 min. This protocol allows for efficient annealing of the primer to its complementary RNA sequence. The primer was extended using Superscript II reverse transcriptase (8 U/µl; BRL) in a buffer containing 50 mM Tris-HCl pH 8.3; 10 mM DTT; 75 mM KCl; 1 mM MgCl₂; 1 mM each dNTP. The extension reaction was carried out at 42°C for 10 min. The reaction was terminated by adding an equal volume of 2x formamide gel loading buffer and freezing on crushed dry ice. The samples were resolved on a 10% polyacrylamide sequencing gel. The primer sequences are as follows: HH primer, 5'-CTCCAGTTTCGAGCTTT-3'; HDV primer, 5'-AAGTAGCCCAGGTCGGACC-3'; HP primer, 5'-ACCAGGTAATATACCACAAC-3'.

As shown in Figure 29, specific bands corresponding to full-length precursor RNA and 3' cleavage products were detected from cells transfected with the self-processing cassettes. All three constructs, in addition to being transcriptionally active, appear to self-process efficiently in the cytoplasm of OST7-1 cells. In particular, the HH and HP(GC) constructs self-process to greater than 95%. The overall extent of self-processing in OST7-1 cells appears to be strikingly similar to the extent of self-processing *in vitro* (Figure 29 "In Vitro +MgCl₂" vs. "Cellular").

Consistent with the *in vitro* self-processing results, the HP(GU) cassette self-processed to approximately 50% in OST7-1 cells. As expected, transfection with plasmids containing the HH(mutant) cassette yielded a primer-extension product corresponding to the full-length RNA with no detectable cleavage products (Figure 29). The latter result strongly suggests that the primer extension band corresponding to the 3' cleavage product is not an artifact of reverse transcription.

Applicant was concerned with the possibility that RNA self-processing might occur during cell lysis, RNA isolation and /or the primer extension assay. Two precautions were taken to exclude this possibility. First, 50 mM EDTA was included in the lysis buffer. EDTA is a strong chelator of divalent

metal ions such as Mg^{2+} and Ca^{2+} that are necessary for ribozyme activity. Divalent metal ions are therefore unavailable to self-processing RNAs following cell lysis. A second precaution involved using primers in the primer-extension assay that were designed to hybridize to essential regions of the processing ribozyme. Binding of these primers should prevent the 3' cis-acting ribozymes from folding into the conformation essential for catalytic activity.

Two experiments were carried out to further eliminate the possibility that self-processing is occurring either during RNA preparations or during the primer extension analysis. The first experiment involves primer extension analysis on full-length precursor RNAs that were added to non-transfected OST7-1 lysates after cell lysis. Thus, only if self-processing is occurring at some point after lysis would cleavage products be detected. Full-length precursor RNAs were prepared by transcribing under conditions of low Mg^{2+} (5 mM) and high NTP concentration (total 12 mM) in an attempt to eliminate the free Mg^{2+} required for the self-processing reaction (Michel et al. 1992 *Genes & Dev.* 6, 1373). The full-length precursor RNAs were gel-purified, and a known amount was added to lysates of non-transfected OST7-1 cells. RNA was purified from these lysates and incubated for 1 hr in DEPC-treated water at 37° C prior to the standard primer extension analysis (*Figure 29, in vitro* "-MgCl₂" control). The predominant RNA detected in all cases corresponds to the primer extension product of full-length precursor RNAs. If, instead, the purified RNA containing the full-length precursor is incubated in 10 mM MgCl₂ prior to the primer extension analysis, most or all of the RNA detected by primer extension analysis undergoes cleavage (*Figure 29, in vitro* "+MgCl₂" control). These results indicate that the standard RNA isolation and primer extension protocols used here do not provide a favorable environment for RNA self-processing, even though the RNA in question is inherently able to undergo self-cleavage.

In a second experiment to demonstrate lack of self-processing during work up, internally-labeled precursor RNAs were prepared and added to non-transfected OST7-1 lysates as in the previous control. The internally-labeled precursor RNAs were carried through the RNA purification and primer extension reactions (in the presence of unlabeled primers) and analyzed to determine the extent of self-processing. By this analysis, the

vast majority of the added full-length RNA remained intact during the entire process of RNA isolation and primer extension.

These two control experiments validate the protocols used and support applicant's conclusion that the self-processing reactions catalyzed by HH, HDV and HP(GC) cassettes are occurring in the cytoplasm of OST7-1 cells.

Sequences in figures 23 through 25 are meant to be non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art.

10 In addition, those in the art will recognize that Applicant provides guidance through the above examples as to how to best design vectors of this invention so that secondary structure of the mRNA allows efficient cleavage by releasing ribozymes. Thus, the specific constructs are not limiting in this invention. Such constructs can be readily tested as
15 described above for such secondary structure, either by computer folding algorithms or empirically. Such constructs will then allow at least 80% completion of release of ribozymes, which can be readily determined as described above or by methods known in the art. That is, any such secondary structure in the RNA does not reduce release of the ribozymes
20 by more than 20%.

IV. Ribozymes Expressed by RNA Polymerase III

Applicant has determined that the level of production of a foreign RNA, using a RNA polymerase III (pol III) based system, can be significantly enhanced by ensuring that the RNA is produced with the 5' terminus and a
25 3' region of the RNA molecule base-paired together to form a stable intramolecular stem structure. This stem structure is formed by hydrogen bond interactions (either Watson-Crick or non-Watson-Crick) between nucleotides in the 3' region (at least 8 bases) and complementary nucleotides in the 5' terminus of the same RNA molecule.

30 Although the example provided below involves a type 2 pol III gene unit, a number of other pol III promoter systems can also be used, for example, tRNA (Hall et al., 1982 *Cell* 29, 3-5), 5S RNA (Nielsen et al., 1993, *Nucleic Acids Res.* 21, 3631-3636), adenovirus VA RNA (Fowlkes and Shenk, 1980 *Cell* 22, 405-413), U6 snRNA (Gupta and Reddy, 1990

Nucleic Acids Res. 19, 2073-2075), vault RNA (Kickoefer et al., 1993 *J. Biol. Chem.* 268, 7868-7873), telomerase RNA (Romero and Blackburn, 1991 *Cell* 67, 343-353), and others.

5 The construct described in this invention is able to accumulate RNA to a significantly higher level than other constructs, even those in which 5' and 3' ends are involved in hairpin loops. Using such a construct the level of expression of a foreign RNA can be increased to between 20,000 and 50,000 copies per cell. This makes such constructs, and the vectors encoding such constructs, excellent for use in decoy, therapeutic editing
10 and antisense protocols as well as for ribozyme formation. In addition, the molecules can be used as agonist or antagonist RNAs (affinity RNAs). Generally, applicant believes that the intramolecular base-paired interaction between the 5' terminus and the 3' region of the RNA should be in a double-stranded structure in order to achieve enhanced RNA
15 accumulation.

Thus, in one preferred embodiment the invention features a pol III promoter system (e.g., a type 2 system) used to synthesize a chimeric RNA molecule which includes tRNA sequences and a desired RNA (e.g., a tRNA-based molecule).

20 The following exemplifies this invention with a type 2 pol III promoter and a tRNA gene. Specifically to illustrate the broad invention, the RNA molecule in the following example has an A box and a B box of the type 2 pol III promoter system and has a 5' terminus or region able to base-pair with at least 8 bases of a complementary 3' end or region of the same RNA
25 molecule. This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using other pol III promoter systems and techniques generally known in the art.

30 By "terminus" is meant the terminal bases of an RNA molecule, ending in a 3' hydroxyl or 5' phosphate or 5' cap moiety. By "region" is meant a stretch of bases 5' or 3' from the terminus that are involved in base-paired interactions. It need not be adjacent to the end of the RNA. Applicant has determined that base pairing of at least one end of the RNA molecule with a region not more than about 50 bases, and preferably only 20 bases, from

the other end of the molecule provides a useful molecule able to be expressed at high levels.

By "3' region" is meant a stretch of bases 3' from the terminus that are involved in intramolecular base-paired interaction with complementary nucleotides in the 5' terminus of the same molecule. The 3' region can be designed to include the 3' terminus. The 3' region therefore is ≥ 0 nucleotides from the 3' terminus. For example, in the S35 construct described in the present invention (Fig. 40) the 3' region is one nucleotide from the 3' terminus. In another example, the 3' region is ~ 43 nt from 3' terminus. These examples are not meant to be limiting. Those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. Generally, it is preferred to have the 3' region within 100 bases of the 3' terminus.

By "tRNA molecule" is meant a type 2 pol III driven RNA molecule that is generally derived from any recognized tRNA gene. Those in the art will recognize that DNA encoding such molecules is readily available and can be modified as desired to alter one or more bases within the DNA encoding the RNA molecule and/or the promoter system. Generally, but not always, such molecules include an A box and a B box that consist of sequences which are well known in the art (and examples of which can be found throughout the literature). These A and B boxes have a certain consensus sequence which is essential for a optimal pol III transcription.

By "chimeric tRNA molecule" is meant a RNA molecule that includes a pol III promoter (type 2) region. A chimeric tRNA molecule, for example, might contain an intramolecular base-paired structure between the 3' region and complementary 5' terminus of the molecule, and includes a foreign RNA sequence at any location within the molecule which does not affect the activity of the type 2 pol III promoter boxes. Thus, such a foreign RNA may be provided at the 3' end of the B box, or may be provided in between the A and the B box, with the B box moved to an appropriate location either within the foreign RNA or another location such that it is effective to provide pol III transcription. In one example, the RNA molecule may include a hammerhead ribozyme with the B box of a type 2 pol III promoter provided in stem II of the ribozyme. In a second example, the B box may be provided in stem IV region of a hairpin ribozyme. A specific example of such RNA molecules is provided below. Those in the art will

recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

By "desired RNA" molecule is meant any foreign RNA molecule which is useful from a therapeutic, diagnostic, or other viewpoint. Such
5 molecules include antisense RNA molecules, decoy RNA molecules, enzymatic RNA, therapeutic editing RNA and agonist and antagonist RNA.

By "antisense RNA" is meant a non-enzymatic RNA molecule that binds to another RNA (target RNA) by means of RNA-RNA interactions and alters the activity of the target RNA (Eguchi et al., 1991 *Annu. Rev. Biochem.* 60, 631-652). By "enzymatic RNA" is meant an RNA molecule
10 with enzymatic activity (Cech, 1988 *J.American. Med. Assoc.* 260, 3030-3035). Enzymatic nucleic acids (ribozymes) act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic
15 portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA.

By "decoy RNA" is meant an RNA molecule that mimics the natural
20 binding domain for a ligand. The decoy RNA therefore competes with natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently binds HIV *tat* protein, thereby preventing it from binding to TAR sequences encoded in the HIV
25 RNA (Sullenger et al., 1990 *Cell* 63, 601-608). This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

By "therapeutic editing RNA" is meant an antisense RNA that can bind
30 to its cellular target (RNA or DNA) and mediate the modification of a specific base.

By "agonist RNA" is meant an RNA molecule that can bind to protein receptors with high affinity and cause the stimulation of specific cellular pathways.

By "antagonist RNA" is meant an RNA molecule that can bind to cellular proteins and prevent it from performing its normal biological function (for example, see Tsai et al., 1992 *Proc. Natl. Acad. Sci. USA* 89, 8864-8868).

- 5 In other aspects, the invention includes vectors encoding RNA molecules as described above, cells including such vectors, methods for producing the desired RNA, and use of the vectors and cells to produce this RNA.

- 10 Thus, the invention features a transcribed non-naturally occurring RNA molecule which includes a desired therapeutic RNA portion and an intramolecular stem formed by base-pairing interactions between a 3' region and complementary nucleotides at the 5' terminus in the RNA. The stem preferably includes at least 8 base pairs, but may have more, for example, 15 or 16 base pairs.

- 15 In preferred embodiments, the 5' terminus of the chimeric tRNA includes a portion of the precursor molecule of the primary tRNA molecule, of which ≥ 8 nucleotides are involved in base-pairing interaction with the 3' region; the chimeric tRNA contains A and B boxes; natural sequences 3' of the B box are deleted, which prevents endogenous RNA processing; the
20 desired RNA molecule is at the 3' end of the B box; the desired RNA molecule is between the A and the B box; the desired RNA molecule includes the B box; the desired RNA molecule is selected from the group consisting of antisense RNA, decoy RNA, therapeutic editing RNA, enzymatic RNA, agonist RNA and antagonist RNA; the molecule has an
25 intramolecular stem resulting from a base-paired interaction between the 5' terminus of the RNA and a complementary 3' region within the same RNA, and includes at least 8 bases; and the 5' terminus is able to base pair with at least 15 bases of the 3' region.

- 30 In most preferred embodiments, the molecule is transcribed by a RNA polymerase III based promoter system, e.g., a type 2 pol III promoter system; the molecule is a chimeric tRNA, and may have the A and B boxes of a type 2 pol III promoter separated by between 0 and 300 bases; DNA vector encoding the RNA molecule of claim 51.

In other related aspects, the invention features an RNA or DNA vector encoding the above RNA molecule, with the portions of the vector encoding the RNA functioning as a RNA pol III promoter; or a cell containing the vector ; or a method to provide a desired RNA molecule in a cell, by
5 introducing the molecule into a cell with an RNA molecule as described above. The cells can be derived from animals, plants or human beings.

In order for RNA-based gene therapy approaches to be effective, sufficient amounts of the therapeutic RNA must accumulate in the appropriate intracellular compartment of the treated cells. Accumulation is
10 a function of both promoter strength of the antiviral gene, and the intracellular stability of the antiviral RNA. Both RNA polymerase II (pol II) and RNA polymerase III (pol III) based expression systems have been used to produce therapeutic RNAs in cells (Sarver & Rossi, 1993 *AIDS Res. & Human Retroviruses* 9, 483-487; Yu et al., 1993 *P.N.A.S.(USA)* 90, 6340-
15 6344). However, pol III based expression cassettes are theoretically more attractive for use in expressing antiviral RNAs for the following reasons. Pol II produces messenger RNAs located exclusively in the cytoplasm, whereas pol III produces functional RNAs found in both the nucleus and the cytoplasm. Pol II promoters tend to be more tissue restricted, whereas pol
20 III genes encode tRNAs and other functional RNAs necessary for basic "housekeeping" functions in all cell types. Therefore, pol III promoters are likely to be expressed in all tissue types. Finally, pol III transcripts from a given gene accumulate to much greater levels in cells relative to pol II genes.

25 Intracellular accumulation of therapeutic RNAs is also dependent on the method of gene transfer used. For example, the retroviral vectors presently used to accomplish stable gene transfer, integrate randomly into the genome of target cells. This random integration leads to varied expression of the transferred gene in individual cells comprising the bulk
30 treated cell population. Therefore, for maximum effectiveness, the transferred gene must have the capacity to express therapeutic amounts of the antiviral RNA in the entire treated cell population, regardless of the integration site.

Pol III System

The following is just one non-limiting example of the invention. A pol III based genetic element derived from a human tRNA^{met} gene and termed $\Delta 3-5$ (Fig. 33; Adeniyi-Jones et al., 1984 *supra*), has been adapted to express antiviral RNAs (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523). This element was inserted into the DC retroviral vector (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523) to accomplish stable gene transfer, and used to express antisense RNAs against moloney murine leukemia virus and anti-HIV decoy RNAs (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523; Sullenger et al., 1990 *Cell* 63, 601-608; Sullenger et al., 1991 *J. Virol.* 65, 6811-6816; Lee et al., 1992 *The New Biologist* 4, 66-74). Clonal lines are expanded from individual cells present in the bulk population, and therefore express similar amounts of the therapeutic RNA in all cells. Development of a vector system that generates therapeutic levels of therapeutic RNA in all treated cells would represent a significant advancement in RNA based gene therapy modalities.

Applicant examined hammerhead (HHI) ribozyme (RNA with enzymatic activity) expression in human T cell lines using the $\Delta 3-5$ vector system (These constructs are termed " $\Delta 3-5$ /HHI"; Fig. 34). On average, ribozymes were found to accumulate to less than 100 copies per cell in the bulk T cell populations. In an attempt to improve expression levels of the $\Delta 3-5$ chimera, the applicant made a series of modified $\Delta 3-5$ gene units containing enhanced promoter elements to increase transcription rates, and inserted structural elements to improve the intracellular stability of the ribozyme transcripts (Fig. 34). One of these modified gene units, termed S35, gave rise to more than a 100-fold increase in ribozyme accumulation in bulk T cell populations relative to the original $\Delta 3-5$ /HHI vector system. Ribozyme accumulation in individual clonal lines from the pooled T cell populations ranged from 10 to greater than 100 fold more than those achieved with the original $\Delta 3-5$ /HHI version of this vector.

The S35 gene unit may be used to express other therapeutic RNAs including, but not limited to, ribozymes, antisense, decoy, therapeutic editing, agonist and antagonist RNAs. Application of the S35 gene unit would not be limited to antiviral therapies, but also to other diseases, such as cancer, in which therapeutic RNAs may be effective. The S35 gene unit may be used in the context of other vector systems besides retroviral

vectors, including but not limited to, other stable gene transfer systems such as adeno-associated virus (AAV; Carter, 1992 *Curr. Opin. Genet. Dev.* 3, 74), as well as transient vector systems such as plasmid delivery and adenoviral vectors (Berkner, 1988 *BioTechniques* 6, 616-629).

- 5 As described below, the S35 vector encodes a truncated version of a tRNA wherein the 3' region of the RNA is base-paired to complementary nucleotides at the 5' terminus, which includes the 5' precursor portion that is normally processed off during tRNA maturation. Without being bound by any theory, Applicant believes this feature is important in the level of
10 expression observed. Thus, those in the art can now design equivalent RNA molecules with such high expression levels. Below are provided examples of the methodology by which such vectors and tRNA molecules can be made.

Δ 3-5 Vectors

- 15 The use of a truncated human tRNA^{met} gene, termed Δ 3-5 (Fig. 33; Adeniyi-Jones et al., 1984 *supra*), to drive expression of antisense RNAs, and subsequently decoy RNAs (Sullenger et al., 1990 *supra*) has recently been reported. Because tRNA genes utilize internal pol III promoters, the antisense and decoy RNA sequences were expressed as chimeras
20 containing tRNA^{met} sequences. The truncated tRNA genes were placed into the U3 region of the 3' moloney murine leukemia virus vector LTR (Sullenger et al., 1990 *supra*).

Base-Paired Structures

- Since the Δ 3-5 vector combination has been successfully used to
25 express inhibitory levels of both antisense and decoy RNAs, applicant cloned ribozyme-encoding sequences (termed as " Δ 3-5/HHI") into this vector to explore its utility for expressing therapeutic ribozymes. However, low ribozyme accumulation in human T cell lines stably transduced with this vector was observed (Fig. 35). To try and improve accumulation of the
30 ribozyme, applicant incorporated various RNA structural elements (Fig. 34) into one of the ribozyme chimeras (Δ 3-5/HHI).

Two strategies were used to try and protect the termini of the chimeric transcripts from exonucleolytic degradation. One strategy involved the incorporation of stem-loop structures into the termini of the transcript. Two

such constructs were cloned, S3 which contains a stem-loop structure at the 3' end, and S5 which contains stem-loop structures at both ends of the transcript (Figure 34). The second strategy involved modification of the 3' terminal sequences such that the 5' terminus and the 3' end sequences can form a stable base-paired stem. Two such constructs were made: S35 in which the 3' end was altered to hybridize to the 5' leader and acceptor stem of the tRNA^{met} domain, and S35Plus which was identical to S35 but included more extensive structure formation within the non-ribozyme portion of the $\Delta 3-5$ chimeras (Figure 34). These stem-loop structures are also intended to sequester non-ribozyme sequences in structures that will prevent them from interfering with the catalytic activity of the ribozyme. These constructs were cloned, producer cell lines were generated, and stably-transduced human MT2 (Harada et al., 1985 *supra*) and CEM (Nara & Fischinger, 1988 *supra*) cell lines were established (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). The RNA sequences and structure of S35 and S35 Plus are provided in Figures 40-47.

Referring to Figure 48, there is provided a general structure for a chimeric RNA molecule of this invention. Each N independently represents none or a number of bases which may or may not be base paired. The A and B boxes are optional and can be any known A or B box, or a consensus sequence as exemplified in the figure. The desired nucleic acid to be expressed can be any location in the molecule, but preferably is on those places shown adjacent to or between the A and B boxes (designated by arrows). Figure 49 shows one example of such a structure in which a desired RNA is provided 3' of the intramolecular stem. A specific example of such a construct is provided in Figures 50 and 51.

Example 26: Cloning of $\Delta 3-5$ -Ribozyme Chimera

Oligonucleotides encoding the S35 insert that overlap by at least 15 nucleotides were designed (5' GATCCACTCTGCTGTTCTGTTTTTGA 3' and 5' CGCGTCAAAAACAGAACAGCAGAGTG 3'). The oligonucleotides (10 μ M each) were denatured by boiling for 5 min in a buffer containing 40 mM Tris.HCl, pH8.0. The oligonucleotides were allowed to anneal by snap cooling on ice for 10-15 min.

The annealed oligonucleotide mixture was converted into a double-stranded molecule using Sequenase[®] enzyme (US Biochemicals) in a

buffer containing 40 mM Tris.HCl, pH7.5, 20 mM MgCl₂, 50 mM NaCl, 0.5 mM each of the four deoxyribonucleotide triphosphates, 10 mM DTT. The reaction was allowed to proceed at 37°C for 30 min. The reaction was stopped by heating to 70°C for 15 min.

- 5 The double stranded DNA was digested with appropriate restriction endonucleases (*Bam*HI and *Mlu*I) to generate ends that were suitable for cloning into the Δ 3-5 vector.

- 10 The double-stranded insert DNA was ligated to the Δ 3-5 vector DNA by incubating at room temperature (about 20°C) for 60 min in a buffer containing 66 mM Tris.HCl, pH 7.6, 6.6 mM MgCl₂, 10 mM DTT, 0.066 μ M ATP and 0.1U/ μ l T4 DNA Ligase (US Biochemicals).

- 15 Competent *E. coli* bacterial strain was transformed with the recombinant vector DNA by mixing the cells and DNA on ice for 60 min. The mixture was heat-shocked by heating to 37°C for 1 min. The reaction mixture was diluted with LB media and the cells were allowed to recover for 60 min at 37°C. The cells were plated on LB agar plates and incubated at 37°C for ~ 18 h.

- 20 Plasmid DNA was isolated from an overnight culture of recombinant clones using standard protocols (Ausubel et al., *Curr. Protocols Mol. Biology* 1990, Wiley & Sons, NY).

The identity of the clones were determined by sequencing the plasmid DNA using the Sequenase[®] DNA sequencing kit (US Biochemicals).

- 25 The resulting recombinant Δ 3-5 vector contains the S35 sequence. The HHI encoding DNA was cloned into this Δ 3-5-S35 containing vector using *Sac*II and *Bam*HI restriction sites.

Example 27: Northern analysis

- 30 RNA from the transduced MT2 cells were extracted and the presence of Δ 3-5/ribozyme chimeric transcripts were assayed by Northern analysis (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). Northern analysis of RNA extracted from MT2 transductants showed that Δ 3-5/ribozyme chimeras of appropriate sizes were expressed (Fig. 35.36). In addition, these results demonstrated the relative differences in accumulation among the different constructs (Figure 35.36). The pattern of

expression seen from the $\Delta 3$ -5/HHI ribozyme chimera was similar to 12 other ribozymes cloned into the $\Delta 3$ -5 vector (not shown). In MT-2 cell line, $\Delta 3$ -5/HHI ribozyme chimeras accumulated, on average, to less than 100 copies per cell.

- 5 Addition of a stem-loop onto the 3' end of $\Delta 3$ -5/HHI did not lead to increased $\Delta 3$ -5 levels (S3 in Fig. 35.36). The S5 construct containing both 5' and 3' stem-loop structures also did not lead to increased ribozyme levels (Fig. 35.36).

- 10 Interestingly, the S35 construct expression in MT2 cells was about 100-fold more abundant relative to the original $\Delta 3$ -5/HHI vector transcripts (Fig. 35.36). This may be due to increased stability of the S35 transcript.

Example 28: Cleavage activity

- 15 To assay whether ribozymes transcribed in the transduced cells contained cleavage activity, total RNA extracted from the transduced MT2 T cells were incubated with a labeled substrate containing the HHI cleavage site (Figure 37). Ribozyme activity in all but the S35 constructs, was too low to detect. However, ribozyme activity was detectable in S35-transduced T cell RNA. Comparison of the activity observed in the S35-transduced MT2 RNA with that seen with MT2 RNA in which varying
- 20 amounts of in vitro transcribed S5 ribozyme chimeras, indicated that between 1-3 nM of S35 ribozyme was present in S35-transduced MT2 RNA. This level of activity corresponds to an intracellular concentration of 5,000-15,000 ribozyme molecules per cell.

Example 29: Clonal variation

- 25 Variation in the ribozyme expression levels among cells making up the bulk population was determined by generating several clonal cell lines from the bulk S35 transduced CEM line (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY) and the ribozyme expression and activity levels in the individual clones were measured (Figure 38 and 39).
- 30 All the individual clones were found to express active ribozyme. The ribozyme activity detected from each clone correlated well with the relative amounts of ribozyme observed by Northern analysis. Steady state ribozyme levels among the clones ranged from approximately 1,000 molecules per cell in clone G to 11,000 molecules per cell in clone H (Fig.

38). The mean accumulation among the clones, calculated by averaging the ribozyme levels of the clones, exactly equaled the level measured in the parent bulk population. This suggests that the individual clones are representative of the variation present in the bulk population.

- 5 The fact that all 14 clones were found to express ribozyme indicate that the percentage of cells in the bulk population expressing ribozyme is also very high. In addition, the lowest level of expression in the clones was still more than 10-fold that seen in bulk cells transduced with the original $\Delta 3-5$ vector. Therefore, the S35 gene unit should be much more effective
10 in a gene therapy setting in which bulk cells are removed, transduced and then reintroduced back into a patient.

Example 30: Stability

- Finally, the bulk S35-transduced line, resistant to G418, was propagated for a period of 3 months (in the absence of G418) to determine
15 if ribozyme expression was stable over extended periods of time. This situation mimicks that found in the clinic in which bulk cells are transduced and then reintroduced into the patient and allowed to propagate. There was a modest 30% reduction of ribozyme expression after 3 months. This difference probably arose from cells with varying amount of ribozyme
20 expression and exhibiting different growth rates in the culture becoming slightly more prevalent in the culture. However, ribozyme expression is apparently stable for at least this period of time.

Example 31: Design and construction of TRZ-tRNA Chimera

- A transcription unit, termed TRZ, is designed that contains the S35
25 motif (Figure 52). A desired RNA (e.g. ribozyme) can be inserted into the indicated region of TRZ tRNA chimera. This construct might provide additional stability to the desired RNA. TRZ-A and TRZ-B are non-limiting examples of the TRZ-tRNA chimera.

- Referring to Fig. 53-54, a hammerhead ribozyme targeted to site I
30 (HHITRZ-A; Fig. 53) and a hairpin ribozyme (HPITRZ-A; Fig. 54), also targeted to site I, is cloned individually into the indicated region of TRZ tRNA chimera. The resulting ribozyme transcripts retain full RNA cleavage activity (see for example Fig. 55). Applicant has shown that efficient

expression of these TRZ tRNA chimera can be achieved in mammalian cells.

Besides ribozymes, desired RNAs like antisense, therapeutic editing RNAs, decoys, can be readily inserted into the indicated region of TRZ-tRNA chimera to achieve therapeutic levels of RNA expression in mammalian cells.

Sequences listed in Figures 40-47 and 50 - 54 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (mutations, insertions and deletions) of the above examples can be readily generated using techniques known in the art, are within the scope of the present invention.

Example 32: Ribozyme expression in T cell lines

Ribozyme expression in T cell lines stably-transduced with either a retroviral-based or an Adeno-associated virus (AAV)-based ribozyme expression vector (Figure 56). The human T cell lines MT2 and CEM were transduced with either retroviral or AAV vectors encoding a neomycin selectable marker and a ribozyme (S35/HHI) expressed from pol III met_t tRNA-driven promoter. Cells stably-transduced with the vectors were selectively expanded medium containing the neomycin antibiotic derivative, G418 (0.7 mg/ml). Ribozyme expression in the stable cell lines was then analyzed by Northern analysis. The probe used to detect ribozyme transcripts also cross-hybridized with human met_t tRNA sequences. Referring to Figure 56, S35/HHI RNA accumulates to significant levels in MT2 and CEM cells when transduced with either the retrovirus or the AAV vector.

These are meant to be non-limiting examples, those skilled in the art will recognize that other vectors such as adenovirus vector (Figure 57), plasmid DNA vector, alpha virus vectors and the other derivatives thereof, can be readily generated to deliver the desired RNA, using techniques known in the art and are within the scope of this invention. Additionally, the transcription units can be expressed individually or in multiples using pol II and/or pol III promoters.

References cited herein, as well as Draper WO 93/23569, 94/02495, 94/06331, Sullenger WO 93/12657, Thompson WO 93/04573, and Sullivan

WO 94/04609, and 93/11253 describe methods for use of vectors described herein, and are incorporated by reference herein. In particular these vectors are useful for administration of antisense and decoy RNA molecules.

5 Example 33: Ligated Ribozymes are catalytically active

The ability of ribozymes generated by ligation methods, described in Draper et al., PCT WO 93/23569, to cleave target RNA was tested on either matched substrate RNA (Fig. 58) or long (622 nt) RNA (Fig. 59, 60 and 61).

Matched substrate RNAs were chemically synthesized using solid-phase RNA synthesis chemistry (Scarange et al., 1990 Nucleic Acids Res. 18, 5433-5441). Substrate RNA was 5' end-labeled using [γ - 32 P] ATP and polynucleotide kinase (Curr. Protocols Mol. Biol. 1992, ed. Ausubel et al., Wiley & Sons, NY). Ribozyme reactions were carried out under ribozyme excess conditions (k_{cat}/K_M ; Herschlag and Cech, 1990 Biochemistry 29, 10159-10171). Briefly, ribozyme and substrate RNA were denatured and renatured separately by heating to 90°C and snap cooling on ice for 10 min in a buffer containing 50 mM Tris. HCl pH 7.5 and 10 mM MgCl₂. Cleavage reaction was initiated by mixing the ribozyme with the substrate at 37°C. Aliquots of 5 μ l were taken at regular intervals of time and the reaction was stopped by mixing with equal volume of formamide gel loading buffer (Curr. Protocols Mol. Biol. 1992, ed. Ausubel et al., Wiley & Sons, NY). The samples were resolved on 20 % polyacrylamide-urea gel. Referring to Fig. 58, $-\Delta G$ refers to the free energy of binding calculated for base-paired interactions between the ribozyme and the substrate RNA (Turner and Sugimoto, 1988 Supra). RPI A is a HH ribozyme with 6/6 binding arms. This ribozyme was synthesized chemically either as a one piece ribozyme or was synthesized in two fragments followed by ligation to generate a one piece ribozyme. The k_{cat}/K_M values for the two ribozymes were comparable.

30 A template containing T7 RNA polymerase promoter upstream of 622 nt long target sequence, was PCR amplified from a DNA clone. The target RNA (containing HH ribozyme cleavage sites B, C and D) was transcribed from this PCR amplified template using T7 RNA polymerase. The transcript was internally labeled during transcription by including [α - 32 P] CTP as one
35 of the four ribonucleotide triphosphates. The transcription mixture was

5 treated with DNase-1, following transcription at 37°C for 2 hours, to digest away the DNA template used in the transcription. RNA was precipitated with Isopropanol and the pellet was washed two times with 70% ethanol to get rid of salt and nucleotides used in the transcription reaction. RNA is resuspended in DEPC-treated water and stored at 4°C. Ribozyme cleavage reactions were carried out under ribozyme excess (k_{cat}/K_M) conditions [Herschlag and Cech 1990 *supra*]. Briefly, 1000 nM ribozyme and 10 nM internally labeled target RNA were denatured separately by heating to 90°C for 2 min in the presence of 50 mM Tris.HCl, pH 7.5 and 10 mM MgCl₂. The RNAs were renatured by cooling to 37°C for 10-20 min. Cleavage reaction was initiated by mixing the ribozyme and target RNA at 37°C. Aliquots of 5 µl were taken at regular intervals of time and the reaction was quenched by adding equal volume of stop buffer. The samples were resolved on a sequencing gel.

15 Example 34: Hammerhead ribozymes with ≥ 2 base-paired stem II are catalytically active

To decrease the cost of chemical synthesis of RNA, applicant was interested in determining whether the length of stem II region of a typical hammerhead ribozyme (≥ 4 bp stem II) can be shortened without decreasing the catalytic efficiency of the HH ribozyme. The length of stem II was systematically shortened by one base-pair at a time. HH ribozymes with three and two base-paired stem II were chemically synthesized using solid-phase RNA phosphoramidite chemistry (Scaringe *et al.*, 1990 *supra*).

25 Matched and long substrate RNAs were synthesized and ribozyme assays were carried out as described in example 33. Referring to figures 62, 63 and 64, data shows that shortening stem II of a hammerhead ribozyme does not significantly alter the catalytic efficiency. It is applicant's opinion that hammerhead ribozymes with ≥ 2 base-paired stem II region are catalytically active.

30 Example 35: Synthesis of catalytically active hairpin ribozymes

RNA molecules were chemically synthesized having the nucleotide base sequence shown in Fig. 65 for both the 5' and 3' fragments. The 3' fragments are phosphorylated and ligated to the 5' fragment essentially as described in example 37. As is evident from the Figure 65, the 3' and 5' fragments can hybridize together at helix 4 and are covalently linked via

GAAA sequence. When this structure hybridizes to a substrate, a ribozyme-substrate complex structure is formed. While helix 4 is shown as 3 base pairs it may be formed with only 1 or 2 base pairs.

- 40 nM mixtures of ligated ribozymes were incubated with 1-5 nM 5' end-labeled matched substrates (chemically synthesized by solid-phase synthesis using RNA phosphoramidite chemistry) for different times in 50 mM Tris/HCl pH 7.5, 10 mM MgCl₂ and shown to cleave the substrate efficiently (Fig.66).

- The target and the ribozyme sequences shown in Fig. 62 and 65 are meant to be non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using other sequences and techniques generally known in the art.

V. Constructs of Hairpin Ribozymes

- There follows an improved trans-cleaving hairpin ribozyme in which a new helix (*i.e.*, a sequence able to form a double-stranded region with another single-stranded nucleic acid) is provided in the ribozyme to base-pair with a 5' region of a separate substrate nucleic acid. This helix is provided at the 3' end of the ribozyme after helix 3 as shown in Figure 3. In addition, at least two extra bases may be provided in helix 2 and a portion of the substrate corresponding to helix 2 may be either directly linked to the 5' portion able to hydrogen bond to the 3' end of the hairpin or may have a linker of at least one base. By trans-cleaving is meant that the ribozyme is able to act in *trans* to cleave another RNA molecule which is not covalently linked to the ribozyme itself. Thus, the ribozyme is not able to act on itself in an intramolecular cleavage reaction.

By "base-pair" is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example Hoogsteen type) of interactions.

- The increase in length of helix 2 of a hairpin ribozyme (with or without helix 5) has several advantages. These include improved stability of the ribozyme-target complex *in vivo*. In addition, an increase in the recognition sequence of the hairpin ribozyme improves the specificity of the ribozyme. This also makes possible the targeting of potential hairpin

ribozyme sites that would otherwise be inaccessible due to neighboring secondary structure.

The increase in length of helix 2 of a hairpin ribozyme (with or without helix 5) enhances *trans*-ligation reaction catalyzed by the ribozyme. *Trans*-ligation reactions catalyzed by the regular hairpin ribozyme (4 bp helix 2) is very inefficient (Komatsu *et al.*, 1993 *Nucleic Acids Res.* 21, 185). This is attributed to weak base-pairing interactions between substrate RNAs and the ribozyme. By increasing the length of helix 2 (with or without helix 5) the rate of ligation (*in vitro* and *in vivo*) can be enhanced several fold.

- 10 Results of experiments suggest that the length of H2 can be 6 bp without significantly reducing the activity of the hairpin ribozyme. The H2 arm length variation does not appear to be sequence dependent. HP ribozymes with 6 bp H2 have been designed against five different target RNAs and all five ribozymes efficiently cleaved their cognate target RNA.
- 15 Additionally, two of these ribozymes were able to successfully inhibit gene expression (e.g., TNF- α) in mammalian cells. Results of these experiments are shown below.

- HP ribozymes with 7 and 8 bp H2 are also capable of cleaving target RNA in a sequence-specific manner, however, the rate of the cleavage reaction is lower than those catalyzed by HP ribozymes with 6 bp H2.

Example 36: 4 and 6 base pair H2

Referring to Figures 67-72, HP ribozymes were synthesized as described above and tested for activity. Surprisingly, those with 6 base pairs in H2 were still as active as those with 4 base pairs.

25 VI. Chemical Modification

Oligonucleotides with 5'-C-alkyl Group

- The introduction of an alkyl group at the 5'-position of a nucleoside or nucleotide sugar introduces an additional center of chirality into the sugar moiety. Referring to Fig. 75, the general structures of 5'-C-alkylnucleotides belonging to the D-allose, 2, and L-talose, 3, sugar families are shown.
- 30 The family names are derived from the known sugars D-allose and L-talose ($R_1 = CH_3$ in 2 and 3 in Figure 75). Useful specific D-allose and L-talose

nucleotide derivatives are shown in Figure 76, 29-32 and Figure 77, 58-61 respectively.

This invention relates to the use of 5'-C-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 5'-C-alkylnucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 5'-C-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 5'-C-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Such nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 5'-C-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 5'-C-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

Thus, in one aspect, the invention features 5'-C-alkylnucleosides, that is a nucleotide base having at the 5'-position on the sugar molecule an alkyl moiety. In a related aspect, the invention also features 5'-C-alkylnucleotides, and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably

includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above. In preferred embodiments, the sugar of the nucleoside or nucleotide is in an optically pure form, as the talose or allose sugar.

5 Examples of various alkyl groups useful in this invention are shown in Figure 75, where each R_1 group is any alkyl. These examples are not limiting in the invention. Specifically, an "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More
10 preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, $=O$, $=S$, NO_2 or $N(CH_3)_2$, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one
15 carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy,
20 $=O$, $=S$, NO_2 , halogen, $N(CH_3)_2$, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons,
25 more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, $=O$, $=S$, NO_2 or $N(CH_3)_2$, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an
30 aromatic group which has at least one ring having a conjugated π electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an
35 alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring

atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

10 In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 5'-C-alkylnucleotides; *e.g.* enzymatic nucleic acids having a 5'-C-alkylnucleotide; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic
15 molecule with at least one nucleotide having at its 5'-position an alkyl group. In other related aspects, the invention features 5'-C-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

20 The 5'-C-alkyl derivatives of this invention provide enhanced stability to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall activity *in vivo*. Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

25 In another aspect, the invention features a method for conversion of a protected allo sugar to a protected talo sugar. In the method, the protected allo sugar is contacted with triphenyl phosphine, diethylazodicarboxylate, and *p*-nitrobenzoic acid under inversion causing conditions to provide the protected talo sugar. While one example of such conditions is provided
30 below, those in the art will recognize other such conditions. Applicant has found that such conversion allows for ready synthesis of all types of nucleotide bases as exemplified in the figures.

While this invention is applicable to all oligonucleotides, applicant has found that the modified molecules of this invention are particularly useful for
35 enzymatic RNA molecules. Thus, below is provided examples of such

molecules. Those in the art will recognize that equivalent procedures can be used to make other molecules without such enzymatic activity. Specifically, Figure 1 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. This is not to be taken as an indication that the Figure is prior art to the pending claims, or that the art discussed is prior art to those claims. Referring to Figure 1, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 5'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Substitutions of any nucleotide with any of the modified nucleotides shown in Figure 75 are possible.

The following are non-limiting examples showing the synthesis of nucleic acids using 5'-C-alkyl-substituted phosphoramidites and the syntheses of the amidites.

Example 37: Synthesis of Hammerhead Ribozymes Containing 5'-C-Alkyl-nucleotides & Other Modified Nucleotides

The method of synthesis would follow the procedure for normal RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 26-29 and 56-59). These 5'-C-alkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group 1 or Group 2 intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 38: Methyl-2,3-O-Isopropylidene-6-Deoxy- β -D-allofuranoside (4)

A suspension of L-rhamnose (100 g, 0.55 mol), CuSO₄ (120 g) and conc. H₂SO₄ (4.0 mL) in 1.0 L of dry acetone was mixed for 24 h at RT, then filtered. Conc. NH₄OH (5 mL) was added to the filtrate and the newly formed precipitate was filtered. The residue was concentrated *in vacuo*, coevaporated with pyridine (2 x 300 mL), dissolved in pyridine (500 mL) and cooled to 0 °C. A solution of *p*-toluenesulfonylchloride (107 g , 0.56

mmol) in dry DCE (500 mL) was added dropwise over 0.5 h. The reaction mixture was left for 16 h at RT. The reaction was quenched by adding ice-water (0.5 L) and, after mixing for 0.5 h, was extracted with chloroform (0.75 L). The organic layer was washed with H₂O (2 x 500 mL), 10% H₂SO₄ (2 x 300 mL), water (2 x 300 mL), sat. NaHCO₃ (2 x 300 mL), brine (2 x 300 mL), dried over MgSO₄ and evaporated to dryness. The residue (115 g) was dissolved in dry MeOH (1 L) and treated with NaOMe (23.2 g, 0.42 mmol) in MeOH. The reaction mixture was left for 16 h at 20 °C, neutralized with dry CO₂ and evaporated to dryness. The residue was suspended in chloroform (750 mL), filtered, concentrated to 100 mL and purified by flash chromatography in CHCl₃ to yield 45 g (37%) of compound 4.

Example 39: Methyl-2,3-O-Isopropylidene-5-O-*t*-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (5).

To solution of methylfuranoside 4 (12.5 g 62.2 mmol) and AgNO₃ (21.25 g, 125.0 mmol) in dry DMF (300 mL) *t*-butyldiphenylsilyl chloride (22.2 g, 81 mmol) was added dropwise under Ar over 0.5 h. The reaction mixture was stirred for 4 h at RT, diluted with CHCl₃ (200 mL), filtered and evaporated to dryness (below 40 °C using a high vacuum oil pump). The residue was dissolved in CH₂Cl₂ (300 mL) washed with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL), dried over MgSO₄ and evaporated to dryness. The residue was purified by flash chromatography in CH₂Cl₂ to yield 20.0 g (75%) of compound 5.

Example 40: Methyl-5-O-*t*-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (6).

Methylfuranoside 5 (13.5 g, 30.6 mmol) was dissolved in CF₃COOH:dioxane:H₂O / 2:1:1 (v/v/v, 200 mL) and stirred at 24 °C for 45 m. The reaction mixture was cooled to -10 °C, neutralized with conc. NH₄OH (140 mL) and extracted with CH₂Cl₂ (500 mL). The organic layer was separated, washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL), dried over MgSO₄ and evaporated to dryness. The product 6 was purified by flash chromatography using a 0-10% MeOH gradient in CH₂Cl₂. Yield 9.0 g (76%).

Example 41: Methyl-2,3-di-O-Benzoyl-5-O-t-Butyldiphenylsilyl-6-Deoxy- β -D-Allofuranoside (7).

Methylfuranoside 6 (7.0 g, 17.5 mmol) was coevaporated with pyridine (2 x 100 mL) and dissolved in pyridine (100 mL). Benzoyl chloride (5.4 g, 38.5 mmol) was added and the reaction mixture was left at RT for 16 h. Dry EtOH (50 mL) was added and the reaction mixture was evaporated to dryness after 0.5 h. The residue was dissolved in CH₂Cl₂ (300 mL), washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL) dried over MgSO₄ and evaporated to dryness. The product was purified by flash chromatography in CH₂Cl₂ to yield 9.5 g (89%) of compound 7.

Example 42: 1-O-Acetyl-2,3-di-O-benzoyl-5-O-t-Butyldiphenylsilyl-6-Deoxy- β -D-Allofuranose (8).

Dibenzoate 7 (4.7 g, 7.7 mmol) was dissolved in a mixture of AcOH (10.0 mL), Ac₂O (20.0 mL) and EtOAc (30 mL) and the reaction mixture was cooled 0 °C. 98% H₂SO₄ (0.15 mL) was then added. The reaction mixture was kept at 0 °C for 16 h, and then poured into a cold 1:1 mixture of sat. NaHCO₃ and EtOAc (150 mL). After 0.5 h of vigorous stirring the organic phase was separated, washed with brine (2 x 75 mL), dried over MgSO₄, evaporated to dryness and coevaporated with toluene (2 x 50 mL). The product was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 4.0 g (82% as a mixture of α and β isomers).

Example 43: 1-(2',3'-di-O-Benzoyl-5'-O-t-Butyldiphenylsilyl-6'-Deoxy- β -D-Allofuranosyl)uracil (9).

Uracil (1.44 g, 11.5 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (3 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT, evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (6.36 g, 10.0 mmol) in dry CH₃CN (100 mL), followed by CF₃SO₃SiMe₃ (2.8 g, 12.6 mmol). The reaction mixture was kept at 24 °C for 16 h, concentrated to 1/3 of its original volume, diluted with 100 mL of CH₂Cl₂ and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄, and evaporated to dryness. The product 9 was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 5.7 g (80%).

Example 44: *N*⁴-Benzoyl-1-(2',3'-Di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy- β -D-Allofuranosyl)Cytosine (10).

*N*⁴-benzoylcytosine (1.84 g, 8.56 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (3 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (3.6 g, 5.6 mmol) in dry CH₃CN (100 mL), followed by CF₃SO₃SiMe₃ (4.76 g, 21.4 mmol). The reaction mixture was boiled under reflux for 5 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. Purification by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ yielded 1.8 g (55%) of compound 10.

Example 45: *N*⁶-Benzoyl-9-(2',3'-di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy- β -D-Allofuranosyl)adenine (11).

*N*⁶-benzoyladenine (2.86 g, 11.86 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (7 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (3.6 g, 5.6 mmol) in dry CH₃CN (100 mL) followed by CF₃SO₃SiMe₃ (6.59 g, 29.7 mmol). The reaction mixture was boiled under reflux for 8 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. The product 11 was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 2.7 g (60%).

Example 46: *N*²-Isobutyryl-9-(2',3'-di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy- β -D-Allofuranosyl)guanine (12).

*N*²-Isobutyrylguanine (1.47 g, 11.2 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (6 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a

- solution of acetates **8** (3.4 g, 5.3 mmol) in dry CH₃CN (100 mL) followed by CF₃SO₃SiMe₃ (6.22 g, 28.0 mmol). The reaction mixture was boiled under reflux for 8 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL),
5 brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. The product **12** was purified by flash chromatography using a gradient of 0-2% MeOH in CH₂Cl₂. Yield: 2.1g (54%).

Example 47: N⁶-Benzoyl-9-(2',3'-di-O-benzoyl-6'-Deoxy-β-D-Allofuranosyl)adenine (15).

- 10 Nucleoside **11** (1.65 g, 2.0 mmol) was dissolved in THF (50 mL) and a 1 M solution of TBAF in THF (4 mL) was added. The reaction mixture was kept at RT for 4 h, evaporated to dryness and the product purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ to yield 1.0 g (85%) of compound **15**.

- 15 Example 48: N⁶-Benzoyl-9-(2',3'-di-O-Benzoyl-5'-O-Dimethoxytrityl-6'-Deoxy-β-D-Allofuranosyl)adenine (19).

- Nucleoside **15** (0.55 g, 0.92 mmol) was dissolved in dry CH₂Cl₂ (50 mL). AgNO₃ (0.34 g, 2.0 mmol), dimethoxytrityl chloride (0.68 g, 2.0 mmol) and sym-collidine (0.48 g) were added under Ar. The reaction mixture was
20 stirred for 2h, diluted with CH₂Cl₂ (100 mL), filtered, evaporated to dryness and coevaporated with toluene (2 x 50 mL). Purification by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ yielded 0.8 g (97%) of compound **19**.

- 25 Example 49: N⁶-Benzoyl-9-(-5'-O-Dimethoxytrityl-6'-Deoxy-β-D-Allofuranosyl)adenine (23).

- Nucleoside **19** (1.8 g, 2 mmol) was dissolved in dioxane (50 mL), cooled to 0 °C and 2 M NaOH (50 mL) was added. The reaction mixture was kept at 0 °C for 45 m, neutralized with Dowex 50 (Pyr⁺ form), filtered and the resin was washed with MeOH (2 x 50 mL). The filtrate was then
30 evaporated to dryness. Purification by flash chromatography using a gradient of 0-10% MeOH in CH₂Cl₂ yielded 1.1 g (80%) of **23**.

Example 50: N⁶-Benzoyl-9-(-5'-O-Dimethoxytrityl-2'-O-*t*-butyldimethylsilyl-6'-Deoxy-β-D-Allofuranosyl)adenine (27).

Nucleoside 23 (1.2 g, 1.8 mmol) was dissolved in dry THF (50 mL). Pyridine (0.50 g, 8 mmol) and AgNO₃ (0.4 g, 2.3 mmol) were added. After
5 the AgNO₃ dissolved (1.5 h), *t*-butyldimethylsilyl chloride (0.35 g, 2.3 mmol) was added and the reaction mixture was stirred at RT for 16 h. The reaction mixture was diluted with CH₂Cl₂ (100 mL), filtered into sat. NaHCO₃ (50 mL), extracted, the organic layer washed with brine (2 x 50 mL), dried over MgSO₄ and evaporated to dryness. The product 27 was
10 purified by flash chromatography using a hexanes:EtOAc / 7:3 gradient. Yield: 0.7 g (50%).

Example 51: N⁶-Benzoyl-9-(-5'-O-Dimethoxytrityl-2'-O-*t*-butyldimethylsilyl-6'-Deoxy-β-D-Allofuranosyl)adenine-3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (31).

15 Standard phosphitylation of 27 according to Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidite 31 in 73% yield.

Example 52: Methyl-5-O-*p*-Nitrobenzoyl-2,3-O-Isopropylidene-6-deoxy-β-L-Tallofuranoside (5)

20 Methylfuranoside 4 (3.1 g 14.2 mmol) was dissolved in dry dioxane (200 mL), *p*-nitrobenzoic acid (10.0 g, 60 mmol) and triphenylphosphine (15.74 g, 60.0 mmol) were added followed by DEAD (10.45 g, 60.0 mmol). The reaction mixture was left at RT for 16 h, EtOH (5 mL) was added, and after 0.5 h the reaction mixture was evaporated to dryness. The residue
25 was dissolved in CH₂Cl₂ (300 mL) washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL) dried over MgSO₄ and evaporated to dryness. Purification by flash chromatography using a hexanes:EtOAc / 9:1 gradient yielded 4.1 g (78%) of compound 33. Subsequent debenzoylation (NaOMe/MeOH) and silylation (see preparation of 5) led to L-talofuranoside 34 which was converted to phosphoramidites 58-61 using
30 the same methodology as described above for the preparation of the phosphoramidites of the D-allo-isomers 29-32.

The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage

or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan et al., PCT WO 94/ 02595.

- 5 The ribozymes and the target RNA containing site O were synthesized, deprotected and purified as described above. RNA cleavage assay was carried out at 37°C in the presence of 10 mM MgCl₂ as described above.

Applicant has substituted 5'-C-Me-L-talo nucleotides at positions A6, A9, A9 + G10, C11.1 and C11.1 + G10, as shown in Figure 78 (HH-O1 to HH-O5). HH-O 1,2,4 and 5 showed almost wild type activity (Figure 79). However, HH-O3 demonstrated low catalytic activity. Ribozymes HH-O1, 2, 3, 4 and 5 are also extremely resistant to degradation by human serum nucleases.

15 Oligonucleotides with 2'-Deoxy-2'-Alkyl nucleotide

This invention uses 2'-deoxy-2'-alkyl nucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 2'-deoxy-2'-alkyl nucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 2'-deoxy-2'-alkyl nucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 2'-deoxy-2'-alkyl nucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Contrary to the findings of De Mesmaeker *et al.* applicant has found that such nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 2'-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair

forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 2'-deoxy-2'-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

Thus, in one aspect, the invention features 2'-deoxy-2'-alkylnucleotides, that is a nucleotide base having at the 2'-position on the sugar molecule an alkyl moiety and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

Examples of various alkyl groups useful in this invention are shown in Figure 81, where each R group is any alkyl. The term "alkyl" does not include alkoxy groups which have an "-O-alkyl" group, where "alkyl" is defined as described above, where the O is adjacent the 2'-position of the sugar molecule.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-deoxy-2'-alkylnucleotides (preferably not a 2'-alkyl- uridine or thymidine); *e.g.* enzymatic nucleic acids having a 2'-deoxy-2'-alkylnucleotide; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group. In other related aspects, the invention features 2'-deoxy-2'-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-alkyl derivatives of this invention provide enhanced stability to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall

activity *in vivo*. Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

5 In another aspect, the invention features hammerhead motifs having enzymatic activity having ribonucleotides at locations shown in Figure 80 at 5, 6, 8, 12, and 15.1, and having substituted ribonucleotides at other positions in the core and in the substrate binding arms if desired. (The term "core" refers to positions between bases 3 and 14 in Figure 80, and the binding arms correspond to the bases from the 3'-end to base 15.1, and 10 from the 5'-end to base 2). Applicant has found that use of ribonucleotides at these five locations in the core provide a molecule having sufficient enzymatic activity even when modified nucleotides are present at other sites in the motif. Other such combinations of useful ribonucleotides can be determined as described by Usman *et al. supra*.

15 Figure 80 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. This is not to be taken as an indication that the Figure is prior art to the pending claims, or that the art discussed is prior art to those claims. Referring to Figure 80 the preferred sequence of a hammerhead ribozyme 20 in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 2'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Although substitutions of any nucleotide with any of the modified nucleotides shown in Figure 81 are 25 possible, and were indeed synthesized, the basic structure composed of primarily 2'-O-Me nucleotides with selected substitutions was chosen to maintain maximal catalytic activity (Yang *et al. Biochemistry* 1992, 31, 5005-5009 and Paoletta *et al. , EMBO J.* 1992, 11, 1913-1919) and ease of synthesis, but is not limiting to this invention.

30 Ribozymes from Figure 80 and Table 45 were synthesized and assayed for catalytic activity and nuclease resistance. With the exception of entries 8 and 17, all of the modified ribozymes retained at least 1/10 of the wild-type catalytic activity. From Table 45, all 2'-modified ribozymes showed very large and significant increases in stability in human serum 35 (shown) and in the other fluids described below (Example 55, data not shown). The order of most aggressive nuclease activity was fetal bovine

serum, > human serum > human plasma > human synovial fluid. As an overall measure of the effect of these 2'-substitutions on stability and activity, a ratio β was calculated (Table 45). This β value indicated that all modified ribozymes tested had significant, >100 - >1700 fold, increases in overall stability and activity. These increases in β indicate that the lifetime of these modified ribozymes *in vivo* are significantly increased which should lead to a more pronounced biological effect.

More general substitutions of the 2'-modified nucleotides from Figure 81 also increased the $t_{1/2}$ of the resulting modified ribozymes. However the catalytic activity of these ribozymes was decreased > 10-fold.

In Figure 86 compound 37 may be used as a general intermediate to prepare derivatized 2'-C-alkyl phosphoramidites, where X is CH₃, or an alkyl, or other group described above.

The following are non-limiting examples showing the synthesis of nucleic acids using 2'-C-alkyl substituted phosphoramidites, the syntheses of the amidites, their testing for enzymatic activity and nuclease resistance.

Example 53: Synthesis of Hammerhead Ribozymes Containing 2'-Deoxy-2'-Alkyl nucleotides & Other 2'-Modified Nucleotides

The method of synthesis used generally follows the procedure for normal RNA synthesis as described in Usman, N.; Ogilvie, K.K.; Jiang, M.-Y.; Cedergren, R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 10, 12, 17, 22, 31, 18, 26, 32, 36 and 38). Other 2'-modified phosphoramidites were prepared according to: 3 & 4, Eckstein *et al.* *International Publication* No. WO 92/07065; and 5 Kois *et al.* *Nucleosides & Nucleotides* 1993, 12, 1093-1109. The average stepwise coupling yields were ~98%. The 2'-substituted phosphoramidites were incorporated into hammerhead ribozymes as shown in Figure 80. However, these 2'-alkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense

oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 54: Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-
5 end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched
on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions
were 1 mM, 200 nM, 40 nM or 8 nM and the final substrate RNA
concentrations were ~ 1 nM. Total reaction volumes were 50 µL. The
assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were
10 initiated by mixing substrate and ribozyme solutions at t = 0. Aliquots of 5
µL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each time
point was quenched in formamide loading buffer and loaded onto a 15%
denaturing polyacrylamide gel for analysis. Quantitative analyses were
performed using a phosphorimager (Molecular Dynamics).

15 Example 55: Stability Assay

500 pmol of gel-purified 5'-end-labeled ribozymes were precipitated
in ethanol and pelleted by centrifugation. Each pellet was resuspended in
20 µL of appropriate fluid (human serum, human plasma, human synovial
fluid or fetal bovine serum) by vortexing for 20 s at room temperature. The
20 samples were placed into a 37 °C incubator and 2 µL aliquots were
withdrawn after incubation for 0, 15, 30, 45, 60, 120, 240 and 480 m.
Aliquots were added to 20 µL of a solution containing 95% formamide and
0.5X TBE (50 mM Tris, 50 mM borate, 1 mM EDTA) to quench further
nuclease activity and the samples were frozen until loading onto gels.
25 Ribozymes were size-fractionated by electrophoresis in 20%
acrylamide/8M urea gels. The amount of intact ribozyme at each time point
was quantified by scanning the bands with a phosphorimager (Molecular
Dynamics) and the half-life of each ribozyme in the fluids was determined
by plotting the percent intact ribozyme vs the time of incubation and
30 extrapolation from the graph.

Example 56: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-O-Phenoxythio-
carbonyl-Uridine (7)

To a stirred solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-
uridine, 6, (15.1 g, 31 mmol, synthesized according to *Nucleic Acid*

Chemistry, ed. Leroy Townsend, 1986 pp. 229-231) and dimethylamino-pyridine (7.57 g, 62 mmol) a solution of phenylchlorothionoformate (5.15 mL, 37.2 mmol) in 50 mL of acetonitrile was added dropwise and the reaction stirred for 8 h. TLC (EtOAc:hexanes / 1:1) showed disappearance of the starting material. The reaction mixture was evaporated, the residue dissolved in chloroform, washed with water and brine, the organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica gel with EtOAc:hexanes / 2:1 as eluent to give 16.44 g (85%) of 7.

10 Example 57: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl-Uridine (8)

To a refluxing, under argon, solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-O-phenoxythiocarbonyl-uridine, 7, (5 g, 8.03 mmol) and allyltributyltin (12.3 mL, 40.15 mmol) in dry toluene, benzoyl peroxide
15 (0.5 g) was added portionwise during 1 h. The resulting mixture was allowed to reflux under argon for an additional 7-8 h. The reaction was then evaporated and the product 8 purified by flash chromatography on silica gel with EtOAc:hexanes / 1:3 as eluent. Yield 2.82 g (68.7%).

Example 58: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine (9)

20 A solution of 8 (1.25 g, 2.45 mmol) in 10 mL of dry tetrahydrofuran (THF) was treated with a 1 M solution of tetrabutylammoniumfluoride in THF (3.7 mL) for 10 m at room temperature. The resulting mixture was evaporated, the residue was loaded onto a silica gel column, washed with 1 L of chloroform, and the desired deprotected compound was eluted with
25 chloroform:methanol / 9:1. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched
30 with methanol (20 mL), evaporated, dissolved in chloroform, washed with 5% aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel, EtOAc:hexanes / 1:1 as eluent, to give 0.85 g (57%) of 9 as a white foam.

Example 59: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (10)

5'-O-Dimethoxytrityl-2'-C-allyl-uridine (0.64 g, 1.12 mmol) was dissolved in dry dichloromethane under dry argon. N,N-Diisopropylethylamine (0.39 mL, 2.24 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.35 mL, 1.57 mmol) was added dropwise to the stirred reaction solution and stirring was continued for 2 h at RT. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using a gradient of 10-60% EtOAc in hexanes containing 1% triethylamine mixture as eluent. Yield: 0.78 g (90%), white foam.

Example 60: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl-N⁴-Acetyl-Cytidine (11)

Triethylamine (6.35 mL, 45.55 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (5.66 g, 81.99 mmol) and phosphorous oxychloride (0.86 mL, 9.11 mmol) in 50 mL of anhydrous acetonitrile. To the resulting suspension a solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-C-allyl uridine (2.32 g, 4.55 mmol) in 30 mL of acetonitrile was added dropwise and the reaction mixture was stirred for 4 h at room temperature. The reaction was concentrated *in vacuo* to a minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The resulting foam was dissolved in 50 mL of 1,4-dioxane and treated with 29% aq. NH₄OH overnight at room temperature. TLC (chloroform:methanol / 9:1) showed complete conversion of the starting material. The solution was evaporated, dried by coevaporation with anhydrous pyridine and acetylated with acetic anhydride (0.52 mL, 5.46 mmol) in pyridine overnight. The reaction mixture was quenched with methanol, evaporated, the residue was dissolved in chloroform, washed with sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, evaporated to dryness and purified by flash chromatography on silica gel (3% MeOH in chloroform). Yield 2.3 g (90%) as a white foam.

Example 61: 5'-O-Dimethoxytrityl-2'-C-Allyl-N⁴-Acetyl-Cytidine

This compound was obtained analogously to the uridine derivative 9 in 55% yield.

Example 62: 5'-O-Dimethoxytrityl-2'-C-allyl-N⁴-Acetyl-Cytidine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (12)

2'-O-Dimethoxytrityl-2'-C-allyl-N⁴-acetyl cytidine (0.8 g, 1.31 mmol) was dissolved in dry dichloromethane under argon. N,N-Diisopropylethylamine (0.46 mL, 2.62 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.38 mL, 1.7 mmol) was added dropwise to a stirred reaction solution and stirring was continued for 2 h at room temperature. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using chloroform:ethanol / 98:2 with 2% triethylamine mixture as eluent. Yield: 0.91 g (85%), white foam.

Example 63: 2'-Deoxy-2'-Methylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine 14 (Hansske,F.; Madej,D.; Robins,M. J. *Tetrahedron* 1984, 40, 125 and Matsuda,A.; Takenuki,K.; Tanaka,S.; Sasaki,T.; Ueda,T. *J. Med. Chem.* 1991, 34, 812) (2.2 g, 4.55 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-uridine (1.0 g, 3.3 mmol, 72.5%) was eluted with 20% MeOH in CH₂Cl₂.

Example 64: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine (15)

2'-Deoxy-2'-methylene-uridine (0.91 g, 3.79 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes as eluant to yield 15 (0.43 g, 0.79 mmol, 22%).

Example 65: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (17)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.43 g, 0.8 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.28 mL, 1.6 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.25 mL, 1.12 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.3 g, 0.4 mmol, 50%) was purified by flash column chromatography over silica gel using a 25-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.42 (CH₂Cl₂: MeOH / 15:1)

Example 66: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine **14** (1.92 g, 12.6 mmol) and triphenylphosphine (2.5 g, 9.25 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol, 70%) eluted with 25% hexanes in EtOAc.

Example 67: 2'-Deoxy-2'-Difluoromethylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol, 68%) was eluted with 20% MeOH in CH₂Cl₂.

Example 68: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine (16)

2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.42 g, 4.18 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture

was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 40% EtOAc:hexanes as eluant to yield 5'-O-DMT-2'-deoxy-2'-difluoromethylene-uridine **16** (1.05 g, 1.8 mmol, 45%).

Example 69: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (**18**)

10 1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.577 g, 1 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.36 mL, 2 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.44 mL, 1.4 mmol). The reaction mixture
15 was stirred for 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.404 g, 0.52 mmol, 52%) was purified by flash chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂: MeOH / 15:1).

20 Example 70: 2'-Deoxy-2'-Methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-4-N-Acetyl-Cytidine **20**

Triethylamine (4.8 mL, 34 mmol) was added to a solution of POCl₃ (0.65 mL, 6.8 mmol) and 1,2,4-triazole (2.1 g, 30.6 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) uridine **19** (1.65 g, 3.4 mmol) in acetonitrile (20 mL) was
25 added dropwise to the above reaction mixture and left to stir at room temperature for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved
30 in dioxane (10 mL) and aq. ammonia (20 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (3 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5 mL). The mixture was concentrated *in vacuo*, dissolved in
35 CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The

organic extracts were dried over Na_2SO_4 , concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine **20** (1.3 g, 2.5 mmol, 73%) was eluted with 20% EtOAc in hexanes.

5 Example 71: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl- β -D-ribofuranosyl)-4-N-Acetyl-Cytosine **21**

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine **20** (1.3 g, 2.5 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol, 80%) was eluted with 10% MeOH in CH_2Cl_2 . 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.81 g, 2.4 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL) and washed with sat. NaHCO_3 (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO_4 , concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield **21** (0.88 g, 1.5 mmol, 75%).

Example 72: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl- β -D-ribofuranosyl)-4-N-Acetyl-Cytosine 3'-(2-Cyanoethyl-N,N-diisopropylphosphoramidite) (**22**)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl- β -D-ribofuranosyl)-4-N-acetyl-cytosine **21** (0.88 g, 1.5 mmol) dissolved in dry CH_2Cl_2 (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.8 mL, 4.5 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at room temperature and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product **22** (0.82 g, 1.04 mmol, 69%) was purified by flash chromatography over silica gel using 50-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.36 (CH_2Cl_2 :MeOH / 20:1).

Example 73: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyl
disiloxane-1,3-diyl)-4-N-Acetyl-Cytidine (24)

Et₃N (6.9 mL, 50 mmol) was added to a solution of POCl₃ (0.94 mL, 10 mmol) and 1,2,4-triazole (3.1 g, 45 mmol) in acetonitrile (20 mL) at 0 °C.

5 A solution of 2'-deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine 23 ([described in example 14] 2.6 g, 5 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at RT for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The

10 organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved in dioxane (20 mL) and aq. ammonia (30 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (5 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat.

15 NaHCO₃ (5mL). The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9

20 mmol, 78%) was eluted with 20% EtOAc in hexanes.

Example 74: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-
ribofuranosyl)-4-N-Acetyl-Cytosine (25)

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9 mmol) dissolved in THF (20 mL) was

25 treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol, 72%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol) was dissolved in

30 pyridine (10 mL) and a solution of DMT-Cl (1.03 g, 3.1 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine

35 (50 mL). The organic extracts were dried over MgSO₄, concentrated *in*

vacuo and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield 25 (1.2 g, 1.9 mmol, 68%).

5 Example 75: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetylcytosine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (26)

10 1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-N-acetylcytosine 25 (0.6 g, 0.97 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.5 mL, 2.9 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product 26, a white foam (0.52 g, 0.63 mmol, 65%) was purified by flash chromatography over silica gel using 30-70% EtOAc gradient in hexanes, 15 containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂:MeOH / 20:1).

Example 76: 2'-Keto-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine (28)

20 Acetic anhydride (4.6 mL) was added to a solution of 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (Brown, J.; Christodolou, C.; Jones, S.; Modak, A.; Reese, C.; Sibanda, S.; Ubasawa A. *J. Chem. Soc. Perkin Trans. I* 1989, 1735) (6.2 g, 9.2 mmol) in DMSO (37 mL) and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated *in vacuo*. The residue was taken up in EtOAc and washed with water. The organic layer was dried over MgSO₄ and 25 concentrated *in vacuo*. The residue was purified on a silica gel column to yield 2'-keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 28 (4.8 g, 7.2 mmol, 78%).

Example 77: 2'-Deoxy-2'-methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)

30 Under a pressure of argon, sec-butyllithium in hexanes (11.2 mL, 14.6 mmol) was added to a suspension of triphenylmethylphosphonium iodide (7.07 g, 17.5 mmol) in THF (25 mL) cooled at -78 °C. The homogeneous orange solution was allowed to warm to -30 °C and a solution of 2'-keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine

- 28 (4.87 g, 7.3 mmol) in THF (25 mL) was transferred to this mixture under argon pressure. After warming to RT, stirring was continued for 24 h. THF was evaporated and replaced by CH₂Cl₂ (250 mL), water was added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl.
- 5 The organic layer was washed with H₂O (20 mL), 5% aqueous NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give the crude compound, which was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 29 (3.86 g, 5.8 mmol, 79%).
- 10

Example 78: 2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine

- 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (3.86 g, 5.8 mmol) dissolved in THF (30 mL)
- 15 was treated with 1 M TBAF in THF (15 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (1.8 g, 4.3 mmol, 74%) was eluted with 10% MeOH in CH₂Cl₂.

- 20 Example 79: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)

- 2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (0.75 g, 1.77 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.66 g, 1.98 mmol) in pyridine (10 mL) was added dropwise over 15 m.
- 25 The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as an eluant to yield 29 (0.81 g, 1.1 mmol, 62%).
- 30

Example 80: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (31)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-6-N-(4-*t*-butylbenzoyl)-adenine 29 dissolved in dry CH₂Cl₂ (15 mL) was placed

in a round bottom flask under Ar. Diisopropylethylamine was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite. The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was
5 evaporated to a syrup *in vacuo* (40 °C). The product was purified by flash chromatography over silica gel using 30-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant (0.7 g, 0.76 mmol, 68%). *R*_f 0.45 (CH₂Cl₂: MeOH / 20:1)

10 Example 81: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 28 (6.7 g, 10 mmol) and triphenylphosphine (2.9 g, 11 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium
15 chlorodifluoroacetate (2.3 g, 15 mmol) in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-
20 (4-*t*-butylbenzoyl)-adenosine (4.1g, 6.4 mmol, 64%) eluted with 15% hexanes in EtOAc.

Example 82: 2'-Deoxy-2'-Difluoromethylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (4.1 g, 6.4 mmol) dissolved in THF
25 (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-6-N-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol, 77%) was eluted
30 with 20% MeOH in CH₂Cl₂.

Example 83: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine (30)

2'-Deoxy-2'-difluoromethylene-6-N-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in

pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The
5 organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as eluant to yield 30 (2.6 g, 3.41 mmol, 69%).

Example 84: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-N-(4-*t*-Butyl-benzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite)
10 (32)

1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-6-*N*-(4-*t*-butylbenzoyl)-adenine 30 (2.6 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (25 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the
15 dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (1.06 mL, 4.76 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). 32 (2.3 g, 2.4 mmol, 70%) was purified by flash column chromatography over silica gel using 20-50% EtOAc gradient in
20 hexanes, containing 1% triethylamine, as eluant. R_f 0.52 (CH₂Cl₂: MeOH / 15:1).

Example 85: 2'-Deoxy-2'-Methoxycarbonylmethylidene-3',5'-*O*-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine (33)

Methyl(triphenylphosphoranylidene)acetate (5.4 g, 16 mmol) was
25 added to a solution of 2'-keto-3',5'-*O*-(tetraisopropyl disiloxane-1,3-diyl)-uridine 14 in CH₂Cl₂ under argon. The mixture was left to stir at RT for 30 h. CH₂Cl₂ (100 mL) and water were added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H₂O (20 mL), 5% aq. NaHCO₃ (20 mL), H₂O to neutrality, and
30 brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give crude product, that was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methoxycarbonylmethylidene-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-uridine 33 (5.8 g, 10.8 mmol, 67.5%).

Example 86: 2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (34)

Et₃N•3 HF (3 mL) was added to a solution of 2'-deoxy-2'-methoxycarboxymethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5 g, 9.3 mmol) dissolved in CH₂Cl₂ (20 mL) and Et₃N (15 mL). The
5 resulting mixture was evaporated *in vacuo* after 1 h and chromatographed on a silica gel column eluting 2'-deoxy-2'-methoxycarbonylmethylidene-uridine **34** (2.4 g, 8 mmol, 86%) with THF:CH₂Cl₂ / 4:1.

Example 87: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (35)

10 2'-Deoxy-2'-methoxycarbonylmethylidene-uridine **34** (1.2 g, 4.02 mmol) was dissolved in pyridine (20 mL). A solution of DMT-Cl (1.5 g, 4.42 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken
15 up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 2-5% MeOH in CH₂Cl₂ as an eluant to yield 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine **35** (2.03 g, 3.46 mmol, 86%).

20 Example 88: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (36)

1-(2'-Deoxy-2'-2'-methoxycarbonylmethylidene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uridine **35** (2.0 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine
25 (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.91 mL, 4.08 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) **36** (1.8 g, 2.3 mmol, 67%)
30 was purified by flash column chromatography over silica gel using a 30-60% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.44 (CH₂Cl₂:MeOH / 9.5:0.5).

Example 89: 2'-Deoxy-2'-Carboxymethylidene-3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-Uridine 37

2'-Deoxy-2'-methoxycarbonylmethylidene-3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-uridine **33** (5.0 g, 10.8 mmol) was dissolved in MeOH
5 (50 mL) and 1 N NaOH solution (50 mL) was added to the stirred solution at RT. The mixture was stirred for 2 h and MeOH removed *in vacuo*. The pH of the aqueous layer was adjusted to 4.5 with 1N HCl solution, extracted with EtOAc (2 x 100 mL), washed with brine, dried over MgSO₄ and concentrated *in vacuo* to yield the crude acid. 2'-Deoxy-2'-
10 carboxymethylidene-3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-uridine **37** (4.2 g, 7.8 mmol, 73%) was purified on a silica gel column using a gradient of 10-15% MeOH in CH₂Cl₂.

The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage
15 or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan *et al.* PCT WO 94/02595.

Oligonucleotides with 3' and/or 5' Dihalophosphonate

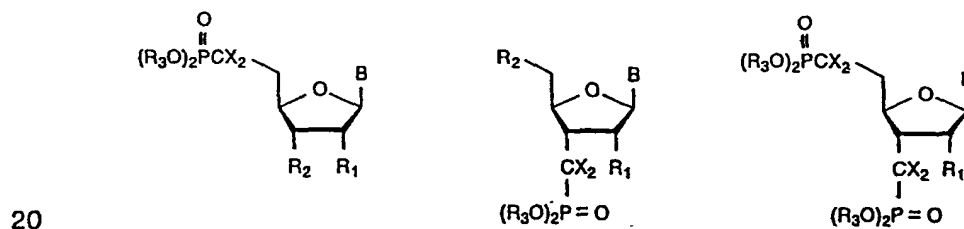
20 This invention synthesis and uses 3' and/or 5' dihalophosphonate-, e.g., 3' or 5'-CF₂-phosphonate-, substituted nucleotides that maintain or enhance the catalytic activity and/or nuclease resistance of an enzymatic or antisense molecule.

As the term is used in this application, 5'- and/or 3'-
25 dihalophosphonate nucleotide containing ribozymes, deoxyribozymes (see Usman *et al.*, PCT/US94/11649, incorporated by reference herein), and chimeras of nucleotides, are catalytic nucleic molecules that contain 5'- and/or 3'-dihalophosphonate nucleotide components replacing, but not limited to, double-stranded stems, single-stranded "catalytic core"
30 sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be
35 targeted to virtually any RNA or DNA transcript. This invention concerns

nucleic acids formed of standard nucleotides or modified nucleotides, which also contain at least one 5'-dihalophosphonate and/or one 3'-dihalophosphonate group.

The synthesis of 1-O-Ac-2,3-di-O-Bz-D-ribofuranose 5-d-
 5+di-halomethylphosphonate in three steps from 1-O-methyl-2,3-O-isopropylidene-β-D-ribofuranose 5-deoxy-5-di-halomethylphosphonate is described (e.g., for the difluoro, in Figure 87). Condensation of this suitably derivatized sugar with silylated pyrimidines and purines affords novel nucleoside 5'-deoxy-5'-di-halomethylphosphonates. These intermediates
 10 may be incorporated into catalytic or antisense nucleic acids by either chemical (conversion of the nucleoside 5'-deoxy-5'-di-halomethylphosphonates into suitably protected phosphoramidites 12a or solid supports 12b, e.g., Figure 88) or enzymatic means (conversion of the nucleoside 5'-deoxy-5'-di-halomethylphosphonates into their
 15 triphosphates, e.g., 14 Figure 89, for T7 transcription).

Thus, in one aspect the invention features 5' and/or 3'-dihalonucleotides and nucleic acids containing such 5' and/or 3'-dihalonucleotides. The general structure of such molecules is shown below.



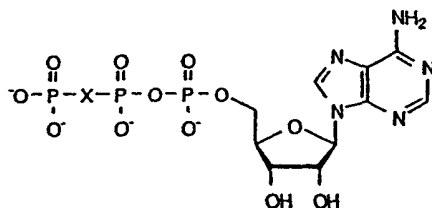
where R₁ is H, OH, or R, where R is a hydroxyl protecting group, e.g., acyl, alkylsilyl, or carbonate; each R₂ is separately H, OH, or R; each R₃ is separately a phosphate protecting group, e.g., methyl, ethyl, cyanoethyl, p-nitrophenyl, or chlorophenyl; each X is separately any halogen; and each B is any nucleotide base.
 25

The invention in particular features nucleic acid molecules having such modified nucleotides and enzymatic activity. In a related aspect the invention features a method for synthesis of such nucleoside 5'-deoxy-5'-
 30 dihalo and/or 3'-deoxy-3'-dihalophosphonates by condensing a

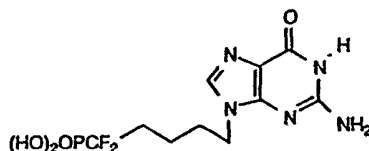
dihalophosphonate-containing sugar with a pyrimidine or a purine under conditions suitable to form a nucleoside 5'-deoxy-5'-dihalophosphonate and/or a 3'-deoxy-3'-dihalophosphonate.

- Phosphonic acids may exhibit important biological properties
- 5 because of their similarity to phosphates (Engel, *Chem. Rev.* 1977, 77, 349-367). Blackburn and Kent (*J. Chem. Soc., Perkin Trans.* 1986, 913-917) indicate that based on electronic and steric considerations _-fluoro and _-difluoromethylphosphonates might mimic phosphate esters better than the corresponding phosphonates. Analogues of pyro- and
- 10 triphosphates 1, where the bridging oxygen atoms are replaced by a difluoromethylene group, have been employed as substrates in enzymatic processes (Blackburn *et al.*, *Nucleosides & Nucleotides* 1985, 4, 165-167; Blackburn *et al.*, *Chem. Scr.* 1986, 26, 21-24). 9-(5,5-Difluoro-5-phosphonopentyl)guanine (2) has been utilized as a multisubstrate
- 15 analogue inhibitor of purine nucleoside phosphorylase (Halazy *et al.*, *J. Am. Chem. Soc.* 1991, 113, 315-317). Oligonucleotides containing methylene groups in place of phosphodiester 5'-oxygens are resistant toward nucleases that cleave phosphodiester linkages between phosphorus and the 5'-oxygen (Breaker *et al.*, *Biochemistry* 1993, 32,
- 20 9125-9128), but can still form stable complexes with complementary sequences. Heinemann *et al.* (*Nucleic Acids Res.* 1991, 19, 427-433) found that a single 3'-methylenephosphonate linkage had a minor influence on the conformation of a DNA octamer double helix.

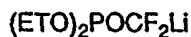
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2



3

- One common synthetic approach to α,α -difluoro-alkylphosphonates features the displacement of a leaving group from a suitable reactive substrate by diethyl (lithiodifluoromethyl)phosphonate (3) (Obayashi *et al.*, *Tetrahedron Lett.* 1982, 23, 2323-2326). However, our attempts to synthesize nucleoside 5'-deoxy-5'-difluoro-methylphosphonates from 5'-deoxy-5'-iodonucleosides using 3 were unsuccessful, *i.e.* starting compounds were quantitatively recovered. The reaction of nucleoside 5'-aldehydes with 3, according to the procedure of Martin *et al.* (Martin *et al.*, *Tetrahedron Lett.* 1992, 33, 1839-1842), led to a complex mixture of products. Recently, the synthesis of sugar α,α -difluoroalkylphosphonates from primary sugar triflates using 3 was described (Berkowitz *et al.*, *J. Org. Chem.* 1993, 58, 6174-6176). Unfortunately, our experience is that nucleoside 5'-triflates are too unstable to be used in these syntheses.
- The following are non-limiting examples showing the synthesis of nucleoside 5'-deoxy-5'-difluoromethyl-phosphonates. Those in the art will recognize that equivalent methods can be readily devised based upon

these examples. These examples demonstrate that it is possible to achieve synthesis of 5'-deoxy-5'-difluoro derivatives in good yield and thus guide those in the art to such equivalent methods. The examples also indicate utility of such synthesis to provide useful oligonucleotides as described above.

Those in the art will recognize that useful modified enzymatic nucleic acids can now be designed, much as described by Draper et al., PCT/US94/13129 hereby incorporated by reference herein (including drawings).

10 Example 90: Synthesis of Nucleoside 5'-Deoxy-5'-difluoromethylphosphonates

Referring to Fig. 87, we synthesized a suitable glycosylating agent from the known D-ribose α,α -difluoromethylphosphonate (4) (Martin et al., *Tetrahedron Lett.* 1992, 33, 1839-1842) which served as a key intermediate for the synthesis of nucleoside 5'-difluoromethylphosphonates.

Methyl 2,3-O-isopropylidene- β -D-ribofuranose α,α -difluoromethylphosphonate (4) was synthesized from the 5-aldehyde according to the procedure of Martin et al. (*Tetrahedron Lett.* 1992, 33, 1839-1842) (Figure 87). Removal of the isopropylidene group was accomplished under mild conditions (I_2 -MeOH, reflux, 18 h (Szarek et al., *Tetrahedron Lett.* 1986, 27, 3827) or Dowex 50 WX8 (H^+), MeOH, RT (about 20-25°C), 3 days) in 72% yield. The anomeric mixture thus obtained was benzoylated with benzoyl chloride/pyridine to afford the 2,3-di-O-benzoyl derivative, which was subjected to mild acetolysis conditions (Walczak et al., *Synthesis*, 1993, 790-792) (Ac_2O , AcOH, H_2SO_4 , EtOAc, 0°C. The desired 1-O-acetyl-2,3-di-O-benzoyl-D-ribofuranose difluoromethylphosphonate (5) was obtained in quantitative yield as an anomeric mixture. These derivatives were used for selective glycosylation of silylated uracil and N^4 -acetylcytosine under Vorbrüggen conditions (Vorbrüggen, *Nucleoside Analogs. Chemistry, Biology and Medical Applications*, NATO ASI Series A, 26, Plenum Press, New York, London, 1980; pp. 35-69. The use of $F_3CSO_2OSi(CH_3)_3$ as a glycosylation catalyst is precluded because it is expected to lead to the undesired 1-ethyluracil or 9-ethyladenine byproducts: Podyukova, et al., *Tetrahedron*

Lett. 1987, 28, 3623-3626 and references cited therein) (SnCl_4 as a catalyst, boiling acetonitrile) to yield β -nucleosides (62% **6a**, 75% **6b**). Glycosylation of silylated N^6 -benzoyladenine under the same conditions yielded a mixture of N-9 isomer **6c** and N-7 isomer **7** in 34% and 15% yield, respectively. The above nucleotides were successfully deprotected using trimethylsilylbromide for the cleavage of the ethyl groups, followed by treatment with ammonia-methanol to remove the acyl protecting groups. Nucleoside 5'-deoxy-5'-difluoromethylphosphonates **8** were finally purified on a DEAE Sephadex A-25 (HCO_3^-) column using a 0.01-0.25 M TEAB gradient for elution and obtained as their sodium salts (82% **8a**; 87% **8b**; 82% **8c**).

Selected analytical data: ^{31}P -NMR (^{31}P) and ^1H -NMR (^1H) were recorded on a Varian Gemini 400. Chemical shifts in ppm refer to H_3PO_4 and TMS, respectively. Solvent was CDCl_3 unless otherwise noted. **5**: ^1H δ 8.07-7.28 (m, Bz), 6.66 (d, $J_{1,2}$ 4.5, αH1), 6.42 (s, βH1), 5.74 (d, $J_{2,3}$ 4.9, βH2), 5.67 (dd, $J_{3,2}$ 4.9, $J_{3,4}$ 6.6, βH3), 5.63 (dd, $J_{3,2}$ 6.7, $J_{3,4}$ 3.6, αH3), 5.57 (dd, $J_{2,1}$ 4.5, $J_{2,3}$ 6.7, αH2), 4.91 (m, H4), 4.30 (m, CH_2CH_3), 2.64 (m, CH_2CF_2), 2.18 (s, βAc), 2.12 (s, αAc), 1.39 (m, CH_2CH_3). ^{31}P δ 7.82 (t, $J_{\text{P,F}}$ 105.2), 7.67 (t, $J_{\text{P,F}}$ 106.5). **6a**: ^1H δ 9.11 (s, 1H, NH), 8.01 (m, 11H, Bz, H6), 5.94 (d, $J_{1',2'}$ 4.1, 1H, H1'), 5.83 (dd, $J_{5,6}$ 8.1, 1H, H5), 5.79 (dd, $J_{2',1'}$ 4.1, $J_{2',3'}$ 6.5, 1H, H2'), 5.71 (dd, $J_{3',2'}$ 6.5, $J_{3',4'}$ 6.4, 1H, H3'), 4.79 (dd, $J_{4',3'}$ 6.4, $J_{4',\text{F}}$ 11.6, 1H, H4'), 4.31 (m, 4H, CH_2CH_3), 2.75 (tq, $J_{\text{H,F}}$ 19.6, 2H, CH_2CF_2), 1.40 (m, 6H, CH_2CH_3). ^{31}P δ 7.77 (t, $J_{\text{P,F}}$ 104.0). **8c**: ^{31}P (vs DSS) (D_2O) δ 5.71 (t, $J_{\text{P,F}}$ 87.9).

25 Compound **7** was deacylated with methanolic ammonia yielding the product that showed λ_{max} (H_2O) 271 nm and λ_{min} 233 nm, confirming that the site of glycosylation was N-7.

Example 91: Synthesis of Nucleic Acids Containing Modified Nucleotide Containing Cores

30 The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman *et al.*, *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe *et al.*, *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end **35** (Figure 88 and Janda *et al.*, *Science* 1989, 244:437-440.). These

nucleoside 5'-deoxy-5'-difluoromethylphosphonates may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group 1 or Group 2 introns, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

5 Example 92: Synthesis of Modified Triphosphate

The triphosphate derivatives of the above nucleotides can be formed as shown in Fig. 89, according to known procedures. *Nucleic Acid Chem.*, Leroy B. Townsend, John Wiley & Sons, New York 1991, pp. 337-340; *Nucleotide Analogs*, Karl Heinz Scheit, John Wiley & Sons New York 1980, pp. 211-218.

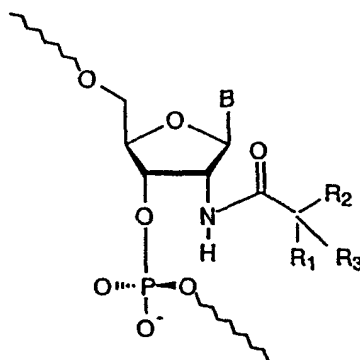
Equivalent synthetic schemes for 3' dihalophosphonates are shown in Figures 90 and 91 using art recognized nomenclature. The conditions can be optimized by standard procedures.

The nucleoside dihalophosphonates described herein are advantageous as modified nucleotides in any nucleic acid structure, e.g., catalytic or antisense, since they are resistant to exo- and endonucleases that normally degrade unmodified nucleic acids *in vivo*. They also do not perturb the normal structure of the nucleic acid in which they are incorporated thereby maintaining any activity associated with that structure. These compounds may also be of use as monomers as antiviral and/or antitumor drugs.

Oligonucleotides with Amido or Peptido Modification

This invention replaces 2'-hydroxyl group of a ribonucleotide moiety with a 2'-amido or 2'-peptido moiety. In other embodiments, the 3' and 5' portions of the sugar of a nucleotide may be substituted, or the phosphate group may be substituted with amido or peptido moieties. Generally, such a nucleotide has the general structure shown in Formula I below:

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FORMULA I

The base (B) is any one of the standard bases or is a modified nucleotide base known to those in the art, or can be a hydrogen group. In addition, either R₁ or R₂ is H or an alkyl, alkene or alkyne group containing between 2 and 10 carbon atoms, or hydrogen, an amine (primary, secondary or tertiary, e.g., R₃NR₄ where each R₃ and R₄ independently is hydrogen or an alkyl, alkene or alkyne having between 2 and 10 carbon atoms, or is a residue of an amino acid, i.e., an amide), an alkyl group, or an amino acid (D or L forms) or peptide containing between 2 and 5 amino acids. The zigzag lines represent hydrogen, or a bond to another base or other chemical moiety known in the art. Preferably, one of R₁, R₂ and R₃ is an H, and the other is an amino acid or peptide.

Applicant has recognized that RNA can assume a much more complex structural form than DNA because of the presence of the 2'-hydroxyl group in RNA. This group is able to provide additional hydrogen bonding with other hydrogen donors, acceptors and metal ions within the RNA molecule. Applicant now provides molecules which have a modified amine group at the 2' position, such that significantly more complex structures can be formed by the modified oligonucleotide. Such modification with a 2'-amido or peptido group leads to expansion and enrichment of the side-chain hydrogen bonding network. The amide and peptide moieties are responsible for complex structural formation of the oligonucleotide and can form strong complexes with other bases, and interfere with standard base pairing interactions. Such interference will allow the formation of a complex nucleic acid and protein conglomerate.

Oligonucleotides of this invention are significantly more stable than existing oligonucleotides and can potentially form biologically active bioconjugates not previously possible for oligonucleotides. They may also be used for *in vitro* selection of unique aptamers, that is, randomly generated oligonucleotides which can be folded into an effective ligand for a target protein, nucleic acid or polysaccharide.

Thus, in one aspect, the invention features an oligonucleotide containing the modified base shown in Formula I, above.

In other aspects, the oligonucleotide may include a 3' or 5' nucleotide having a 3' or 5' located amino acid or aminoacyl group. In all these aspects, as well as the 2'-modified nucleotide, it will be evident that various standard modifications can be made. For example, an "O" may be replaced with an S, the sugar may lack a base (i.e., abasic) and the phosphate moiety may be modified to include other substitutions (see Sproat, *supra*).

Example 93: General procedure for the preparation of 2'-aminoacyl-2'-deoxy-2'-aminonucleoside conjugates.

Referring to Fig. 92, to the solution of 2'-deoxy-2'-amino nucleoside (1 mmol) and N-Fmoc L- (or D-) amino acid (1 mmol) in methanol [dimethylformamide (DMF) and tetrahydrofuran (THF) can also be used], 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) [or 1-isobutyloxycarbonyl-2-isobutyloxy-1,2-dihydroquinoline (IIDQ)] (2 mmol) is added and the reaction mixture is stirred at room temperature or up to 50 °C from 3-48 hours. Solvents are removed under reduced pressure and the residual syrup is chromatographed on the column of silica-gel using 1-10 % methanol in dichloromethane. Fractions containing the product are concentrated yielding a white foam with yields ranging from 85 to 95 %. Structures are confirmed by ¹H NMR spectra of conjugates which show correct chemical shifts for nucleoside and aminoacyl part of the molecule. Further proofs of the structures are obtained by cleaving the aminoacyl protecting groups under appropriate conditions and assigning ¹H NMR resonances for the fully deprotected conjugate.

Partially protected conjugates described above are converted into their 5'-O-dimethoxytrityl derivatives and into 3'-phosphoramidites using standard procedures (Oligonucleotide Synthesis: A Practical Approach,

M.J. Gait ed.; IRL Press, Oxford, 1984). Incorporation of these phosphoramidites into RNA was performed using standard protocols (Usman *et al.*, 1987 *supra*).

A general deprotection protocol for oligonucleotides of the present invention is described in Fig. 93.

The scheme shows synthesis of conjugate of 2'-d-2'-aminouridine. This is meant to be a non-limiting example, and those skilled in the art will recognize that, variations to the synthesis protocol can be readily generated to synthesize other nucleotides (e.g., adenosine, cytidine, guanosine) and/or abasic moieties.

Example 94: RNA cleavage by hammerhead ribozymes containing 2'-aminoacyl modifications.

Hammerhead ribozymes targeted to site N (see Fig. 94) are synthesized using solid-phase synthesis, as described above. U4 and U7 positions are modified, individually or in combination, with either 2'-NH-alanine or 2'-NH-lysine.

RNA cleavage assay *in vitro*: Substrate RNA is 5' end-labeled using [γ -³²P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂. The reaction is initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of time.

Referring to Fig. 95, hammerhead ribozymes containing 2'-NH-alanine or 2'-NH-lysine modifications at U4 and U7 positions cleave the target RNA efficiently.

Sequences listed in Figure 94 and the modifications described in Figure 95 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 95: Aminoacylation of 3'-ends of RNA

- I. Referring to Fig. 96, 3'-OH group of the nucleotide is converted to succinate as described by Gait, *supra*. This can be linked with amino-alkyl solid support (for example: CpG). Zig-zag line indicates linkage of 3'OH group with the solid support.

II. Preparation of aminoacyl-derivatized solid support

A) Synthesis of O-Dimethoxytrityl (O-DMT) amino acids

- Referring to Fig. 97, to a solution of L- (or D-) serine, tyrosine or threonine (2 mmol) in dry pyridine (15 ml) 4,4'-dimethoxytrityl chloride (3 mmol) is added and the reaction mixture is stirred at RT (about 20-25°C) for 16 h. Methanol (10 ml) is then added and the solution evaporated under reduced pressure. The residual syrup was partitioned between 5% aq. NaHCO₃ and dichloromethane, organic layer was washed with brine, dried (Na₂SO₄) and concentrated *in vacuo*. The residue is purified by flash silicagel column chromatography using 2-10% methanol in dichloromethane (containing 0.5 % pyridine). Fractions containing product are combined and concentrated *in vacuo* to yield white foam (75-85 % yield).

B) Preparation of the solid support and its derivatization with amino acids

- Referring to Fig. 97, the modified solid support (has an OH group instead of the standard NH₂ end group) was prepared according to Haralambidis et al., Tetrahedron Lett. 1987, 28, 5199, (P denotes aminopropyl CPG or polystyrene type support). O-DMT or NH-monomethoxytrityl (NH-MMT amino acid was attached to the above solid support using standard procedures for derivatization of the solid support (Gait, 1984, *supra*) creating a base-labile ester bond between amino acids

and the support. This support is suitable for the construction of RNA/DNA chain using suitably protected nucleoside phosphoramidites.

Example 96: Aminoacylation of 5'-ends of RNA

I. Referring to Fig. 98, 5'-amino-containing sugar moiety was synthesized as described (Mag and Engels, 1989 *Nucleic Acids Res.* 17, 5973). Aminoacylation of the 5'-end of the monomer was achieved as described above and RNA phosphoramidite of the 5'-aminoacylated monomer was prepared as described by Usman *et al.*, 1987 *supra*. The phosphoramidite was then incorporated at the 5'-end of the oligonucleotide using standard solid-phase synthesis protocols described above.

II. Referring to Fig. 99, aminoacyl group(s) is attached to the phosphate group at the 5'-end of the RNA using standard procedures described above.

VII. Reversing Genetic Mutations

Modification of existing nucleic acid sequences can be achieved by homologous recombination. In this process a transfected sequence recombines with homologous chromosomal sequences and can replace the endogenous cellular sequence. Boggs, 8 *International J. Cell Cloning* 80, 1990, describes targeted gene modification. It reviews the use of homologous DNA recombination to correct genetic defects. Banga and Boyd, 89 *Proc. Natl. Acad. Sci. U.S.A.* 1735, 1992, describe a specific example of *in vivo* site-directed mutagenesis using a 50 base oligonucleotide. In this methodology a gene or gene segment is essentially replaced by the oligonucleotide used.

This invention uses a complementary oligonucleotide to position a nucleotide base changing activity at a particular site on a gene (RNA or genomic DNA), such that the nucleotide modifying activity will change (or revert) a mutation to wild-type, or its equivalent. By reversion or change of a mutation, we refer to reversion in a broad sense, such as when a mutation at a second site which leads to functional reversion to a wild type phenotype. Also, due to the degeneracy of the genetic code, a revertant may be achieved by changing any one of the three codon positions. Additionally, creation of a stop codon in a deleterious gene (or transcript) is defined here as reverting a mutant phenotype to wild-type. An example of

this type of reversion is creating a stop codon in a critical HIV proviral gene in a human.

Referring to Figures 100 and 101, broadly there are two approaches to causing a site directed change in order to revert a mutation to wild-type.

- 5 In one (Fig. 100) the oligonucleotide is used to target RNA specifically. RNA is provided with a complementary (Watson-crick) oligonucleotide sequence to that in the target molecule. In this case the sequence modifying oligonucleotide would (analogously to an antisense oligonucleotide or ribozyme) have to be continuously present to revert the
- 10 RNA as it is made by the cell. Such a reversion would be transient and would potentially require continuous addition of more sequence modifying oligonucleotide. The transient nature of this approach is an advantage, in that treatment could be stopped by simply removing the sequence modifying oligonucleotide (as with a traditional drug).
- 15 A second approach targets DNA (Fig. 101) and has the advantage that changes may be permanently encoded in the target cell's genetic code. Thus, a single course (or several courses) of treatment may lead to permanent reversion of the genetic disease. If inadvertent chromosomal mutations are introduced this may cause cancer, mutate other genes, or
- 20 cause genetic changes in the germ-line (in patients of reproductive age). However, if the base changing activity is a specific methylation that may modulate gene expression it would not necessarily lead to germ-line transmission. See Lewin, Genes, 1983 John Wiley & Sons, Inc. NY pp 493-496.
- 25 Complementary base pairing to single-stranded DNA or RNA is one method of directing an oligonucleotide to a particular site of DNA. This could occur by a strand displacement mechanism or by targeting DNA when it is single-stranded (such as during replication, or transcription). Another method is using triple-strand binding (triplex formation) to double-
- 30 stranded DNA, which is an established technique for binding polypyrimidine tracts, and can be extended to recognize all 4 nucleotides. See Povsic, T., Strobel, S., & Dervan, P. (1992). Sequence-specific double-strand alkylation and cleavage of DNA mediated by triple-helix formation. J. Am. Chem. Soc. **114**, 5934-5944 (1992). Knorre, D.G., Valentin, V.V.,
- 35 Valentina, F.Z., Lebedev, A.V. & Federova, O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk,

1993) describe conjugation of reactive groups or enzyme to oligonucleotides and can be used in the methods described herein.

5 Recently, antisense oligonucleotides have been used to redirect an incorrect splice into order to obtain correct splicing of a splice mutant globin gene *in vitro*. Dominski Z; Kole R (1993) Restoration of correct splicing in thalassemia pre-mRNA by antisense oligonucleotides. Proc Natl Acad Sci U S A 90:8673-7. Analogously, in one preferred embodiment of this invention a complementary oligomer is used to correct an existing mutant RNA, instead of the traditional approach of inhibiting that RNA by
10 antisense.

In either the RNA or DNA mode, after binding to a particular site on the RNA or DNA the oligonucleotide will modify the nucleic acid sequence. This can be accomplished by activating an endogenous enzyme (see Figure 102), by appropriate positioning of an enzyme (or ribozyme)
15 conjugated (or activated by the duplex) to the oligonucleotide, or by appropriate positioning of a chemical mutagen. Specific mutagens, such as nitrous acid which deaminates C to U, are most useful, but others can also be used if inactivation of a harmful RNA is desired.

RNA editing is an naturally occurring event in mammalian cells in
20 which a sequence modifying activity edits a RNA to its proper sequence post-transcriptionally. Higuchi, M., Single, F., Kohler, M., Sommer, B., and Seeburg, P. (1993) RNA Editing of AMPA Receptor Subunit GluR-B: A base-paired intron-exon structure determines position and efficiency Cell 75:1361-1370. The machinery involved in RNA editing can be co-opted by
25 a suitable oligonucleotide in order to promote chemical modification.

The changes in the base created by the methods of this invention cause a change in the nucleotide sequence, either directly, or after DNA repair by normal cellular mechanisms. These changes functionally correct a genetic defect or introduce a stop codon. Thus, the invention is distinct
30 from techniques in which an active chemical group (*e.g.*, an alkylator) is attached to an antisense or triple strand oligonucleotide in order to chemically inactivate the target RNA or DNA.

Thus, this invention creates an alteration to an existing base in a nucleic acid molecule so that the base is read *in vivo* as a different base.

This includes correcting a sequence instead of inactivating a gene but can also include inactivating a deleterious gene.

Thus, in one aspect, the invention features a method for altering in vivo the nucleotide base sequence of a naturally occurring mutant nucleic acid molecule. The method includes contacting the nucleic acid molecule in vivo with an oligonucleotide or peptide nucleic acid or other sequence specific binding molecules able to form a duplex or triplex molecule with the nucleic acid molecule. After formation of the duplex or triplex molecule a base modifying activity chemically or enzymatically alters the targeted base directly, or after nucleic acid repair *in vivo*. This results in the functional alteration of the nucleic acid sequence.

By "alter", as it is used in this context, is meant that one or more chemical moieties in a targeted base, or bases, is altered so that the mutant nucleic acid will be functionally different. Thus, this is distinct from prior methods of correcting defects in DNA, such as homologous recombination, in which an entire segment of the targeted sequence is replaced with a segment of DNA from the transfected nucleic acid. This is also distinct from other methods that use reactive groups to inactivate a RNA or DNA target, in that this method functionally corrects the sequence of the target, instead of merely damaging it, by causing it to be read by a polymerase as a different base from the original base. As noted above, the naturally occurring enzymes in a cell can be utilized to cause the chemical alteration, examples of which are provided below.

By "functionally alter" is meant that the ability of the target nucleic acid to perform its normal function (*i.e.*, transcription or translation control) is changed. For example, an RNA molecule may be altered so that it can cause production of a desired protein, or a DNA molecule can be altered so that upon DNA repair, the DNA sequence is changed.

By "mutant" it is meant a nucleic acid molecule which is altered in some way compared to equivalent molecules present in a normal individual. Such mutants may be well known in the art, and include, molecules present in individuals with known genetic deficiencies, such as muscular dystrophy, or diabetes and the like. It also includes individuals with diseases or conditions characterized by abnormal expression of a gene, such as cancer, thalassemia's and sickle cell anemia, and cystic

fibrosis. It allows modulation of lipid metabolism to reduce artery disease, treatment of integrated AIDS genomes, and AIDS RNA, and Alzheimer's disease. Thus, this invention concerns alteration of a base in a mutant to provide a "wild type" phenotype and/or genotype. For deleterious conditions this involves altering a base to allow expression or prevent expression as is necessary. When treating an infection, such as HIV, it concerns inactivation of a gene in the HIV RNA by mutation of the mutant (*i.e.*, non-human gene) to a wild type (*i.e.*, no production of a non-human protein). Such modification is performed *in trans* rather than *in cis* as in prior methods.

In preferred embodiments, the oligonucleotide is of a length (at least 12 bases, preferably 17 - 22) sufficient to activate dsRNA deaminase *in vivo* to cause conversion of an adenine base to inosine; the oligonucleotide is an enzymatic nucleic acid molecule that is active to chemically modify a base (see below); the nucleic acid molecule is DNA or RNA; the oligonucleotide includes a chemical mutagen, *e.g.*, the mutagen is nitrous acid; and the oligonucleotide causes deamination of 5-methylcytosine to thymidine, cytosine to uracil, or adenine to inosine, or methylation of cytosine to 5-methylcytosine.

In a most preferred embodiment, the invention features correction of a mutation, rather than inactivation of a target by causing a mutation.

Using *in vitro* directed evolution, it is possible to screen for ribozymes with catalytic activities different than RNA cleavage. Bartel, D. and Szostak, J. (1993) Isolation of new ribozymes from a large pool of random sequences. Science 261:1411-1418. Using these methods of *in vitro* directed evolution, an enzymatic nucleic acid molecule, or ribozyme that mutates bases, instead of cleaving the phosphodiester backbone can be selected. This is a convenient method of obtaining an enzyme with the appropriate base sequence modifying activities for use in the present invention.

Sequence modifying activities can change one nucleotide to another (or modify a nucleotide so that it will be repaired by the cellular machinery to another nucleotide). Sequence modifying activities could also delete or add one or more nucleotides to a sequence. A specific embodiment of adding sequences is described by Sullenger and Cech, PCT/US94/12976

hereby incorporated by reference herein), in which entire exons with wild-type sequence are spliced into a mutant transcript. The present invention features only the addition of a few bases (1 - 3).

Thus, in another aspect, the invention features ribozymes or enzymatic nucleic acid molecules active to change the chemical structure of an existing base in a separate nucleic acid molecule. Applicant is the first to determine that such molecules would be useful, and to provide a description of how such molecules might be isolated.

Molecules used to achieve *in situ* reversion can be delivered using the existing means employed for delivering antisense molecules and ribozymes, including liposomes and cationic lipid complexes. If the *in situ* reverting molecule is composed only of RNA, then expression vectors can be used in a gene therapy protocol to produce the reverting molecules endogenously, analogously to antisense or ribozymes expression vectors. There are several advantages of using such an expression vector, rather than simply replacing the gene through standard gene therapy. Firstly, this approach would limit the production of the corrected gene to cells that already express that gene. Furthermore, the corrected gene would be properly regulated by its natural transcriptional promoter. Lastly, reversion can be used when the mutant RNA creates a dominant gain of function protein (e.g., in sickle cell anemia), where correction of the mutant RNA is necessary to stop the production of the deleterious mutant protein, and allow production of the corrected protein.

Endogenous Mammalian RNA Editing System

It was observed in the mid-1980s that the sequence of certain cellular RNAs were different from the DNA sequence that encodes them. By a process called RNA editing, cellular RNA are post-transcriptionally modified to a) create a translation initiation and termination codons, b) enable tRNA and rRNA to fold into a functional conformation (for a review see Bass, B. L. (1993) In The RNA World, R. Gesteland, R. and Atkins, J. eds. (Cold Spring Harbor, New York; CSH Lab. Press) pp. 383-418). The process of RNA editing includes base modification, deletion and insertion of nucleotides.

Although, the RNA editing process is widespread among lower eukaryotes, very few RNAs (four) have been reported to undergo editing in

mammals (Bass, *supra*). The predominant mode of RNA editing in mammalian system is base modification ($C \rightarrow U$ and $A \rightarrow G$). The mechanism of RNA editing in the mammalian system is postulated to be that $C \rightarrow U$ conversion is catalyzed by cytidine deaminase. The mechanism of conversion of $A \rightarrow G$ has recently been reported for glutamate receptor B subunit (gluR-B) in rat PC12 cells (Higuchi, M. et al. (1993) *Cell* 75, 1361-1370). According to Higuchi gluR-B mRNA precursor attains a structure such that intron 11 and exon 11 can form a stable stem-loop structure. This stem-loop structure is a substrate for a nuclear double strand-specific adenosine deaminase enzyme. The deamination will result in the conversion of $A \rightarrow I$. Reverse transcription followed by double strand synthesis will result in the incorporation of G in place of A.

In the present invention, the endogenous deaminase activity or other such activities can be utilized to achieve targeted base modification.

The following are examples of the invention to illustrate different methods by which *in vivo* conversion of a base can be achieved. These are provided only to clarify specific embodiments of the invention and are not limiting to the invention. Those in the art will recognize that equivalent methods can be readily devised within the scope of the claims.

Example 97: Exploiting cellular dsRNA dependent Adenine to Inosine converter:

An endogenous activity in most mammalian cells and *Xenopus* oocytes converts about 50% of adenines to inosines in double stranded RNA. (Bass, B. L., & Weintraub, H. (1988). An unwinding activity that covalently modifies it double-stranded RNA substrate. *Cell*, 55, 1089-1098.). This activity can be used to cause an *in situ* reversion of a mutation at the RNA level. Referring to Figures 102 and 104, for demonstration purposes a stop codon is incorporated into the coding region of dystrophin, which is fused to the reporter gene luciferase. This stop codon can be reverted by targeting an antisense RNA which is long enough to activate the dsRNA deaminase, which converts Adenines to Inosines. The A to I transition will be read by the ribosome as an A to G transition in some cases and will thereby functionally revert the stop codon. While other A's in this region may be converted to I's and read as G, converting an A to I (G) cannot create a stop codon. The A to I transitions

in the region surrounding the target mutation will create some point mutations, however, the function of the dystrophin protein is rarely inactivated by point mutations.

5 The reverted mRNA was then translated in a cell lysate and assayed for luciferase activity. As evidenced by the dramatic increase in luciferase counts in the graph in figure 103, the A to I transition was read by the ribosome as an A to G transition and the stop codon has successfully been
10 reverted with the lysate treated complex. As a control, an irrelevant non-complementary RNA oligonucleotide was added to the dystrophin/luciferase mRNA. As expected, in this case no translation (luciferase activity) is observed because of the stop codon. As an additional control, the hybrid was not treated with extract, and again no translation (luciferase activity) is observed (Figure 103).

15 While other A's in the targeted region may have been converted to I's and read as G, converting an A to I (G) cannot create a stop codon, so the ribosome will still read through the region. Dystrophin is not generally sensitive to point mutations if the open reading frame is maintained, so a dystrophin protein made from an mRNA reverted by this method should retain full activity.

20 The following detail specifics of the methodology: RNA oligonucleotides were synthesized on a 394 (ABI) synthesizer using phosphoramidite chemistry. The sequence of the synthetic complementary RNA that binds to the mutant dystrophin sequence is as follows (5' to 3'):

25 CCCGCGGTAGATCTTTCTGGAGGCTTACAGTTTTCTACAAACCTCC
CTTCAAA (Seq. ID No. 1)

Referring to Figure 104, fifty-nine base pairs of a human dystrophin mutant sequence containing a stop codon was fused in frame to the luciferase coding region using standard cloning technology, into the *Hind* III and *Not* I sites of pRC-CMV (Invitrogen, San Diego, CA). The AUG of
30 luciferase was deleted. The sequences of the insert from the *Hind* III site to the start of the luciferase coding region is (5' to 3'):

GCCCCTGAGGAGCGATGGAGGCCTTGAAGGGAGGTTTGTGAAAA
CTGTAAGCCTCCAGAAAGATCTACCGCGG (Seq ID No. 2)

This corresponds to base pairs 3649-3708 of normal dystrophin (Entrez ID # 311627) with a *Sac* II site at the 3' end. This plasmid was used as a template for *in vitro* transcription of mRNA using T7 polymerase with the manufacturers protocol (Promega, Madison, WI).

- 5 *Xenopus* nuclear extracts were prepared in 0.5X TGKED buffer (0.5X= 25mM Tris (pH 7.9), 12.5% glycerol, 25 mM KCl, 0.25mM DTT and 0.05mM EDTA), by vortexing nuclei and resuspended in a volume of 0.5X TGKED equal to total cytoplasm volume of the oocytes. Bass, B.L. & Weintraub, H. *Cell* 55, 1089-1098 (1988).
- 10 The target mRNA at 500ng/ul was pre-annealed to 1 micromolar complementary or irrelevant RNA oligonucleotide by heating to 70°C, and allowing it to slowly cool to 37°C over 30 minutes. Fifty nanograms of mRNA pre-annealed to the RNA oligonucleotides was added to 7ul of nuclear extracts containing 1mM ATP, 15mM EDTA, 1600un/ml RNasin
- 15 and 12.5mM Tris pH 8 to a total volume of 12ul. Bass, B.L. & Weintraub, H. *supra*. This mixture, which contains the dsRNA deaminase activity, was incubated for 30 minutes at 25°C. Next, 1.5ul of this mixture was added to a rabbit reticulocyte lysate *in vitro* translation mixture and translated for two hours according to the manufacturers protocol (Life Technologies,
- 20 Gaithersberg, MD), except that an additional 1.3 mM magnesium acetate was added to compensate for the EDTA carried through from the nuclear extract mixture. Luciferase assays were performed on 15ul of extract with the Promega luciferase assay system (Promega, Madison, WI), and luminescence was detected with a 96 well luminometer, and the results are
- 25 displayed in the graph in figure 102.

Example 98: Base changing activities

- The chemical synthesis of antisense and triple-strand forming oligomers conjugated to reactive groups is well studied and characterized (Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova,
- 30 O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk, 1993) and Povsic, T., Strobel, S. & Dervan, P. Sequence-specific double-strand alkylation and cleavage of DNA mediated by triple-helix formation *J. Am. Chem. Soc.* **114**, 5934-5944 (1992). Reactive groups such as alkylators that can modify nucleotide
- 35 bases in targeted RNA or DNA have been conjugated to oligonucleotides.

Additionally enzymes that modify nucleic acids have been conjugated to oligonucleotides. (Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova, O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk, 1993). In the past these

5 conjugated chemical groups or enzymes have been used to inactivate DNA or RNA that is specifically targeted by antisense or triple-strand interactions. Below is a list of useful base changing activities that could be used to change the sequence of DNA or RNA targeted by antisense or triple strand interactions, in order to achieve *in situ* reversion of mutations,

10 as described herein (see figure 100-104).

1. Deamination of 5-methylcytosine to create thymidine (performed by the enzyme cytidine deaminase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993). Also, nitrous acid or related compounds promote oxidative deamination of

15 C to be read at T (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.). Additionally hydroxylamine or related compounds can transform C to be read at T (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.)

20 2. Deamination of cytosine to create uracil (performed by the enzyme cytidine deaminase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993) or by chemical groups similar to nitrous acid that promote oxidative deamination (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.)

25 Boston, 1987, PP.226-230.)

3. Deamination of Adenine to be read like G (Inosine) (as done by the adenosine deaminase, AMP deaminase or the dsRNA deaminating activity (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993).

30 4. Methylation of cytosine to 5-methylcytosine

5. Transforming thymidine (or uracil) to O²-methyl thymidine (or O²-methyl uracil), to be read as cytosine by alkynitrosoureas (Xu, and Swann, Tetrahedron Letters 35:303-306 (1994)).

6. Transforming guanine to 6-O-methyl (or other alkyls) to be read as adenine (Mehta and Ludlum, *Biochimica et Biophysica Acta*, 521:770-778 (1978) which can be done with the mutagen ethyl methane sulfonate (EMS) Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.

7. Amination of uracil to cytosine (as performed by the cellular enzyme CTP synthetase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993).

- The following are examples of useful chemical modifications that can be utilized in the present invention. There are a few preferred straightforward chemical modifications that can change one base to another base. Appropriate mutagenic chemicals are placed on the targetting oligonucleotide, *e.g.*, nitrous acid, or a suitable protein with such activity. Such chemicals and proteins can be attached by standard procedures. These include molecules which introduce fundamental chemical changes, that would be useful independent of the particular technical approach. See Lewin, *Genes*, 1983 John Wiley & Sons, Inc. NY pp 42-48.

- The following matrix shows that the chemical modifications noted can cause transversion reversions (pyrimidine to pyrimidine, or purine to purine) in RNA or DNA. The transversions (pyrimidine to purine, or purine to pyrimidine) are not preferred because these are more difficult chemical transformations. The footnotes refer to the specific desired chemical transformations. The bold footnotes refer to the reaction on the opposite DNA strand. For example, if one desires to change an A to a G, this can be accomplished at the DNA level by using reaction #5 to change a T to a C in the opposing strand. In this example an A/T base pair goes to A/C, then when the DNA is replicated, or mismatch repair occurs this can become G/C, thus the original A has been converted to a G.

30

ISR matrix**Reverted Base**

Mutant base	A	T(U)	C	G
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161

A	-	Transversion	Transversion	DNA ^{5,3} /RNA ³
T(U)	Transversion	-	DNA ⁵ /RNA ⁷	Transversion
C	Transversion	RNA ² /DNA ⁶	-	Transversion
G	DNA ⁶ /RNA ⁶	Transversion	Transversion	-

- 1 Deamination of 5-methylcytosine to create thymidine.
- 2 Deamination of cytosine to create uracil.
- 3 Deamination of Adenine to be read like G (Inosine).
- 5 4 Methylation of cytosine to 5-methylcytosine.
- 5 5 Transforming thymidine (or uracil) to O²-methyl thymidine (or O²-methyl uracil), to be read as cytosine (Xu, and Swann, Tetrahedron Letters 35:303-306 (1994)).
- 10 6 Transforming guanine to 6-O-methyl (or other alkyls) to be read as adenine (Mehta and Ludlum, Biochimica et Biophysica Acta, 521:770-778 (1978)).
7. 7. Amination of uracil to cytosine. Bass *supra*. fig. 6c.

In Vitro Selection Strategy

- Referring to Figure 105, there is provided a schematic describing an approach to selecting for a ribozyme with such base changing activity. An RNA is designed that folds back on itself (this is similar to approaches already used to select for RNA ligases, Bartel, D. and Szostak, J. (1993) Isolation of new ribozymes from a large pool of random sequences. Science 261:1411-1418). A degenerate loop opposing the base to be modified provides for diversity. After incubating this library of molecules in a buffer, the RNA is reverse transcribed into DNA (that is, using standard *in vitro* evolution protocol. Tuerk and Gold, 249 Science 505, 1990) , and then the DNA is selected for having a base change. A restriction enzyme cleavage and size selection or its equivalent is used to isolate the fraction of DNAs with the appropriate base change. The cycle could then be repeated many times.

The *in vitro* selection (evolution) strategy is similar to approaches developed by Joyce (Beaudry, A. A. and Joyce, G.F. (1992) Science 257, 635-641; Joyce, G. F. (1992) Scientific American 267, 90-97) and Szostak (Bartel, D. and Szostak, J. (1993) Science 261:1411-1418; Szostak, J. W. (1993) TIBS 17, 89-93). Briefly, a random pool of nucleic acids is synthesized wherein, each member contains two domains: a) one domain consists of a region with defined (known) nucleotide sequence; b) the second domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to bind to its target (the region flanking the mutant nucleotide), 2) complementary DNA (cDNA) synthesis and PCR amplification of molecules selected for their base modifying activity, 3) introduction of restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides represented at every position within the random region) or the degeneracy can be partial (Beaudry, A. A. and Joyce, G.F. (1992) Science 257, 635-641). In this invention, the degenerate domain is flanked by regions containing known sequences (see Figure 105), such that the degenerate domain is placed across from the mutant base (the base that is targeted for modification). This random library of nucleic acids is incubated under conditions that ensure folding of the nucleic acids into conformations that facilitate the catalysis of base modification (the reaction protocol may also include certain cofactors like ATP or GTP or an S-adenosyl-methionine (if methylation is desired) in order to make the selection more stringent). Following incubation, nucleic acids are converted into complementary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with base modification (at the mutant base position) can be separated from rest of the population of nucleic acids by using a variety of methods. For example, a restriction endonuclease cleavage site can either be created or abolished as a result of base modification. If a restriction endonuclease site is created as a result of base modification, then the library can be digested with the restriction endonuclease (RE). The fraction of the population that is cleaved by the RE is the population that has been able to catalyze the base modification reaction (active pool). A new piece of DNA (containing oligonucleotide primer binding sites for PCR and RE sites for cloning) is ligated to the termini of the active pool to facilitate PCR amplification and subsequent cycles (if necessary) of selection. The final pool of nucleic acids with the best base modifying activity is cloned in to a plasmid vector

and transformed into bacterial hosts. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

Base modifying enzymatic nucleic acids (identified via *in vitro* selection) can be used to cause the chemical modification *in vivo*.

In addition, the ribozyme could be evolved to specifically bind a protein having an enzymatic base changing activity.

Such ribozymes can be used to cause the above chemical modifications *in vivo*. The ribozymes or above noted antisense-type molecules can be administered by methods discussed in the above referenced art.

VIII. Administration of Nucleic Acids

Applicant has determined that double-stranded nucleic acid lacking a transcription termination signal can be used for continuous expression of the encoded RNA. This is achieved by use of an R-loop, *i.e.*, an RNA molecule non-covalently associated with the double-stranded nucleic acid and which causes localized denaturation ("bubble" formation) within the double stranded nucleic acid (Thomas et al., 1976 Proc. Natl. Acad. Sci. USA 73, 2294). In addition, applicant has determined that that the RNA portion of the R-loop can be used to target the whole R-loop complex to a desirable intracellular or cellular site, and aid in cellular uptake of the complex. Further, applicant indicates that expression of enzymatically active RNA or ribozymes can be significantly enhanced by use of such R-loop complexes.

Thus, in one aspect, the invention features a method for introduction of enzymatic nucleic acid into a cell or tissue. A complex of a first nucleic acid encoding the enzymatic nucleic acid and a second nucleic acid molecule is provided. The second nucleic acid molecule has sufficient complementarity with the first nucleic acid to be able to form an R-loop base pair structure under physiological conditions. The R-loop is formed in a region of the first nucleic acid molecule which promotes expression of RNA from the first nucleic acid under physiological conditions. The method further includes contacting the complex with a cell or tissue under

conditions in which the enzymatic nucleic acid is produced within the cell or tissue.

By "complex" is simply meant that the two nucleic acid molecules interact by intermolecular bond formation (such as by hydrogen bonding) between two complementary base-paired sequences. The complex will generally be stable under physiological condition such that it is able to cause initiation of transcription from the first nucleic acid molecule.

The first and second nucleic acid molecules may be formed from any desired nucleotide bases, either those naturally occurring (such as adenine, guanine, thymine and cytosine), or other bases well known in the art, or may have modifications at the sugar or phosphate moieties to allow greater stability or greater complex formation to be achieved. In addition, such molecules may contain non-nucleotides in place of nucleotides. Such modifications are well known in the art, see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science, 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Sproat, B. *European Patent Application* 92110298.4 which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.

By "sufficient complementarity" is meant that sufficient base pairing occurs so that the R-loop base pair structure can be formed under the appropriate conditions to cause transcription of the enzymatic nucleic acid. Those in the art will recognize routine tests by which such sufficient base pairs can be determined. In general, between about 15 - 80 bases is sufficient in this invention.

By "physiological condition" is meant the condition in the cell or tissue to be targeted by the first nucleic acid molecule, although the R-loop complex may be formed under many other conditions. One example is use of a standard physiological saline at 37°C, but it is simply desirable in this invention that the R-loop structure exists to some extent at the site of action so that the expression of the desired nucleic acid will be achieved at that site of action. While it is preferred that the R-loop structure be stable under

those conditions, even a minimal amount of formation of the R-loop structure to cause expression will be sufficient. Those in the art will recognize that measurement of such expression is readily achieved, especially in the absence of any promoter or leader sequence on the first nucleic acid molecule (Daube and von Hippel, 1992 Science 258, 1320).
5 Such expression can thus only be achieved if an R-loop structure is truly formed with the second nucleic acid. If a promoter or leader sequence is provided, then it is preferred that the R-loop be formed at a site distant from those regions so that transcription is enhanced.

10 In a related aspect, the invention features a method for introduction of ribonucleic acid within a cell or tissue by forming an R-loop base-paired structure (as described above) with the first nucleic acid molecule lacking any promoter region or transcription termination signal such that once expression is initiated it will continue until the first nucleic acid is degraded.

15 In another related aspect, the invention features a method in which the second nucleic acid is provided with a localization factor, such as a protein, *e.g.*, an antibody, transferin, a nuclear localization peptide, or folate, or other such compounds well known in the art, which will aid in targeting the R-loop complex to a desired cell or tissue.

20 In preferred embodiments, the first nucleic acid is a plasmid, *e.g.*, one without a promoter or a transcription termination signal; the second nucleic acid is of length between about 40-200 bases and is formed of ribonucleotides at a majority of positions; and the second nucleic acid is covalently bonded with a ligand such as a nucleic acid, protein, peptide,
25 lipid, carbohydrate, cellular receptor, nuclear localization factor, or is attached to maleimide or a thiol group: the first nucleic acid is an expression plasmid lacking a promoter able to express a desired gene, *e.g.*, it is a double-stranded molecule formed with a majority of deoxyribonucleic acids; the R-loop complex is a RNA/DNA heteroduplex;
30 no promoter or leader region is provided in the first nucleic acid; and the R-loop is adapted to prevent nucleosome assembly and is designed to aid recruitment of cellular transcription machinery.

In other preferred embodiments, the first nucleic acid encodes one or more enzymatic nucleic acids, *e.g.*, it is formed with a plurality of

intramolecular and intermolecular cleaving enzymatic nucleic acids to allow release of therapeutic enzymatic nucleic acid *in vivo*.

In a further related aspect, the invention features a complex of the above first nucleic acid molecules and second nucleic acid molecules.

5 R-loop complex

An R-loop complex is designed to provide a non-integrating plasmid so that, when an RNA polymerase binds to the plasmid, transcription is continuous until the plasmid is degraded. This is achieved by hybridizing an RNA molecule, 40 to 200 nucleotides in length, to a DNA expression
10 plasmid resulting in an R-loop structure (see figure 106). This RNA, when conjugated with a ligand that binds to a cell surface receptor, triggers internalization of the plasmid/RNA-ligand complex. Formation of R-loops in general is described by DeWet, 1987 Methods in Enzymol. 145, 235; Neuwald et al., 1977 J. Virol. 21,1019; and Meyer et al., 1986 J. Ult. Mol.
15 Str. Res. 96, 187. Thus, those in the art can readily design complexes of this invention following the teachings of the art.

Promoters placed in retroviral genomes have not always behaved as planned in that the additional promoter will serve as a stop signal or reverses the direction of the polymerase. Applicant was told that creation
20 of an R-loop between the promoter and the reporter gene increased the transfection efficiency. Incubation of an RNA molecule with a double-stranded DNA molecule, containing a region of complementarity with the RNA will result in the formation of a stable RNA-DNA hetroduplex and the DNA strand that has a sequence identical to the RNA will be displaced into
25 a loop-like structure called the R-loop. This displacement of DNA strand occurs because an RNA-DNA duplex is more stable compared to a DNA-DNA duplex. Applicant was also told that an 80 nt long RNA was used to generate a R-loop structure in a plasmid encoding the β -galactosidase gene. The R-loop was initiated either in the promoter region or in the
30 leader sequence. Plasmids containing an R-loop structure were microinjected into the cytoplasm of COS cells and the gene expression was assayed. R-loop formation in the promoter region of the plasmid inhibited expression of the gene. RNA that hybridized to the leader sequence between the promoter and the gene, or directly to the first 80
35 nucleotides of the mRNA increased the expression levels 8-10 fold. The

proposed mechanism is that R-loop formation prevents nucleosome assembly, thus making the DNA more accessible for transcription. Alternatively, the R-loop may resemble a RNA primer promoting either DNA replication or transcription (Daube and von Hippel, 1992, supra).

- 5 One of the salient features of this invention is to generate R-loops in expression vectors of choice and introduce them into cells to achieve enhanced expression from the expression vector. The presence of an R-loop may aid in the recruitment of cellular transcription machinery. Once an RNA polymerase binds to the plasmid and initiates transcription, the
10 process will continue until a termination signal is reached, or the plasmid is degraded.

- This invention will increase the expression of ribozymes inside a cell. The idea is to construct a plasmid with no transcription termination signal, such that a transcript-containing multiple ribozyme units can be
15 generated. In order to liberate unit length ribozymes, self-processing ribozymes can be cloned downstream of each therapeutic ribozyme (see figure 107) as described by Draper *supra*.

Ligand Targeting

- Another salient feature of this invention is that the RNA used to
20 generate R-loop structures can be covalently linked to a ligand (nucleic acid, proteins, peptides, lipids, carbohydrates, *etc.*). Specific ligands can be chosen such that the ligand can bind selectively to a desired cell surface receptor. This ligand-receptor interaction will help internalize a plasmid containing an R-loop. Thus, RNA is used to attach the ligand to the
25 DNA such that localization of the gene to certain regions of the cell is achieved. One of several methods can be used to attach a ligand to RNA. This includes the incorporation of deoxythymidine containing a 6 carbon spacer having a terminal primary amine into the RNA (see figure 108). This amino group can be directly derivatized with the ligand, such as folate (Lee and Low, 1994 J. Biol. Chem. 269, 3198-3204). The RNA containing a 6
30 carbon spacer with a terminal amine group is mixed with folate and the mixture is reacted with activators like 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC). This reaction should be carried out in the presence of 1-Hydroxybenzotriazole hydrate (HOBT) to prevent
35 any undesirable side reactions.

The RNA can also be derivatized with a heterobifunctional crosslinking agent (or linker) like succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB). The SMPB introduces a maleimide into the RNA. This maleimide can then react with a thiol moiety either in a peptide or in a protein. Thiols can also be introduced into proteins or peptides that lack naturally occurring thiols using succinylacetylthioacetate. The amino linker can be attached at the 5' end or 3' end of the RNA. The RNA can also contain a series of nucleotides that do not hybridize to the DNA and extend the linker away from the RNA/DNA complex, thus increasing the accessibility of the ligand for its receptor and not interfering with the hybridization. These techniques can be used to link peptides such as nuclear localization signal (NLS) peptides (Lanford et al., 1984 Cell 37, 801-813; Kalderon et al., 1984 Cell 39, 499-509; Goldfarb et al., 1986 Nature 322, 641-644) and/or proteins like the transferrin (Curiel et al., 1991 Proc. Natl. Acad. Sci. USA 88, 8850-8854; Wagner et al., 1992 Proc. Natl. Acad. Sci. USA 89, 6099-6103; Giulio et al., 1994 Cell. Signal. 6, 83-90) to the ends of R-loop forming RNA in order to facilitate the uptake and localization of the R-loop-DNA complex. To link a protein to the ends of R-loop forming RNA, an intrinsic thiol can be used to react with the maleimide or the thiols can be introduced into the protein itself using either iminothiolate or succinimidyl acetyl thioacetate (SATA; Duncan et al., 1983 Anal. Biochem 132, 68). The SATA requires an additional deprotection step using 0.5 M hydroxylamine.

In addition liposomes can be used to cause an R-loop complex to be delivered to an appropriate intracellular site by techniques well known in the art. For example, pH-sensitive liposomes (Connor and Huang, 1986 Cancer Res. 46, 3431-3435) can be used to facilitate DNA transfection.

Calcium phosphate mediated or electroporation-mediated delivery of the R-loop complex in to desired cells can also be readily accomplished.

In vitro Selection

In vitro selection strategies can be used to select nucleic acids that a) can form stable R-loops b) selectively bind to specific cell surface receptors. These nucleic acids can then be covalently linked to each other. This will help internalize the R-loop-containing plasmid efficiently using receptor-mediated endocytosis. The *in vitro* selection (evolution) strategy is

similar to approaches developed by Joyce (Beaudry and Joyce, 1992 Science 257, 635-641; Joyce, 1992 Scientific American 267, 90-97) and Szostak (Bartel and Szostak, 1993 Science 261:1411-1418; Szostak, 1993 TIBS 17, 89-93). Briefly, a random pool of nucleic acids is synthesized wherein each member contains two domains: a) one domain consists of a region with defined (known) nucleotide sequence; b) the second domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to bind to its target (a specific region of the double strand DNA), 2) complimentary DNA (cDNA) synthesis and PCR amplification of molecules selected for their affinity to form R-loop and/or their ability to bind to a specific receptor, 3) introduction of a restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides represented at every position within the random region) or the degeneracy can be partial (Beaudry and Joyce, 1992 Science 257, 635-641). In this invention, the degenerate domain is flanked by regions containing known sequences. This random library of nucleic acids is incubated under conditions that ensure equilibrium binding to either double-stranded DNA or cell surface receptor. Following incubation, nucleic acids are converted into complementary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with desired characteristics can be separated from the rest of the population of nucleic acids by using a variety of methods (Joyce, 1992 supra). The desired pool of nucleic acids can then be carried through subsequent rounds of selection to enrich the population with the most desired traits. These molecules are then cloned in to appropriate vectors. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

Other embodiments are within the following claims.

TABLE ICharacteristics of Ribozymes**Group I Introns**

Size: ~200 to >1000 nucleotides.

Requires a U in the target sequence immediately 5' of the cleavage site.

Binds 4-6 nucleotides at 5' side of cleavage site.

Over 75 known members of this class. Found in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.

RNAseP RNA (M1 RNA)

Size: ~290 to 400 nucleotides.

RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.

Roughly 10 known members of this group all are bacterial in origin.

Hammerhead Ribozyme

Size: ~13 to 40 nucleotides.

Requires the target sequence UH immediately 5' of the cleavage site.

Binds a variable number nucleotides on both sides of the cleavage site.

14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figures 1 and 2)

Hairpin Ribozyme

Size: ~50 nucleotides.

Requires the target sequence GUC immediately 3' of the cleavage site.

Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent (Figure 3).

Hepatitis Delta Virus (HDV) Ribozyme

Size: 50 - 60 nucleotides (at present).

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Only 1 known member of this class. Found in human HDV (Figure 4).

***Neurospora* VS RNA Ribozyme**

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.
Sequence requirements not fully determined.
Binding sites and structural requirements not fully determined. Only 1
known member of this class. Found in *Neurospora* VS RNA (Figure 5).

Table 2
Human ICAM HH Target sequence

nt. Position	Target Sequences	nt. Position	Target Sequences
11	CCCCAGU C GACGCTG	386	ACCGUGU A CUGGACU
23	CUGAGCU C CUCUGCU	394	CUGGACU C CAGAACG
26	AGCUCCU C UGCUACU	420	CACCCCU C CCCUCUU
31	CUCUGCU A CUCAGAG	425	CUCCCCU C UUGGCAG
34	UGCUCU C AGAGUUG	427	CCCCUCU U GGCAGCC
40	UCAGAGU U GCAACCU	450	AGAACCU U ACCUCAC
48	GCAACCU C AGCCUCG	451	GAACCUU A CCCUACG
54	UCAGCCU C GCUAUGG	456	UUACCCU A CGCUGCC
58	CCUUGCU A UGGCUCC	495	CCAACCU C ACGUGG
64	UAUGGCU C CCAGCAG	510	UGCUGCU C CGUGGGG
96	CCGCACT C CUGGUCC	564	CUGAGGU C ACGACCA
102	UCCUGGU C CUGCUCG	592	GAGAGAU C ACCAUGG
108	UCCUGCU C GGGGCTC	607	AGCCAAU U UCUCGUG
115	CGGGGCU C UGUUCCC	608	GCCAAUU U CUCGUGC
119	GCUCUGU U CCCAGGA	609	CCAAUUU C UCGUGCC
120	CUCUGUU C CCAGGAC	611	AAUUUCU C GUGCCGC
146	CAGACAU C UGUGUCC	656	GAGCUGU U UGAGAAC
152	UCUGUGU C CCCUCA	657	AGCUGUU U GAGAACA
158	UCCCCCU C AAAAGUC	668	AACACCU C GGCCCCC
165	CAAAAGU C AUCCUGC	677	GCCCCCU A CCAGCUC
168	AAGUCAU C CGGCCCC	684	ACCAGCU C CAGACCU
185	GGAGGCU C CGUGCUG	692	CAGACCU U UGUCCUG
209	AGCACCU C CUGUGAC	693	AGACCUU U GUCCUGC
227	CCCAAGU U GUUGGGC	696	CCUUUGU C CUGCCAG
230	AAGUUGU U GGGCAUA	709	AGCGACU C CCCCACA
237	UGGGCAU A GAGACCC	720	CACAACU U GUCAGCC
248	ACCCCGU U GCCUAAA	723	AACUUGU C AGCCCCC
253	GUUGCCU A AAAAGGA	735	CCCGGGU C CUAGAGG
263	AAGGAGU U GCUCCUG	738	GGGUCCU A GAGGUGG
267	AGUUGCU C CUGCCUG	765	CCGUGGU C UGUUCCC
293	AAGGUGU A UGAACUG	769	GGUCUGU U CCCUGGA
319	AGAAGAU A GCCAACC	770	GUCUGUU C CCUGGAC
335	ADGUGCU A UUCAAAC	785	GGGUGU U CCCAGUC
337	GUGCUAU U CAAACUG	786	GGCUGUU C CCAGUCU
338	UGCUAUU C AAACUGC	792	UCCAGU C UCGGAGG
359	GGGCAGU C AACAGCU	794	CCAGUCU C GGAGGCC
367	AACAGCU A AAACCUU	807	CCCAGGU C CACUGG
374	AAAACCU U CCUCACC	833	CAGAGGU U GAACCCC
375	AAACCUU C CUCACCG	846	CCACAGU C ACCUAUG
378	CCUUCU C ACGUGU	851	GUCACCU A UGGCAAC

863	AACGACU C CUUCUUG	1408	UCCGAGU C UUGAGGG
866	GACUCCU U CUUGGCC	1410	GAGAUUU U GAGGGCA
867	ACUCCUU C UUGGCCA	1421	GGCACCU A CCUCUGU
869	UCCUUCU C GGCCAAG	1425	CCUACCU C UGUGGGG
881	AAGGCCU C AGUCAGU	1429	CCUCUGU C GGGCCAG
885	CCUCAGU C AGUGUGA	1444	GAGCACU C AAGGGGA
933	GUGCAGU A ADACUGG	1455	GGGAGGU C ACCCGCG
936	CAGUAAU A CUUGGGA	1482	AUGUGCU C UCCCCCC
978	UGACCAU C UACAGCU	1484	GUGUCU C CCCCCGG
980	ACCAUCU A CAGCUUU	1493	CCCCCGU A UGAGAUU
986	UACAGCU U UCCGGCG	1500	AUGAGAU U GUCAUCA
987	ACAGCUU U CCGGGCG	1503	AGAUGU C AUCAUCA
988	CAGCUUU C CCGGGCC	1506	UUGUCAU C AUCACUG
1005	ACGUGAU U CUGAGGA	1509	UCAUCAU C ACUGUGG
1006	CGUGAUU C UGACGAA	1518	CUGUGGU A GCAGCCG
1023	CAGAGGU C UCAGAAG	1530	CGCAGU C AUAAUGG
1025	GAGGUUU C AGAAGGG	1533	CAGUCAU A AUGGGCA
1066	CCACCCU A GAGCCAA	1551	CAGGCCU C AGCACGU
1092	AUGGGGU U CCGGCC	1559	AGCACGU A CCUCUUA
1093	UGGGGUU C CAGCCCA	1563	CGUACCU C UAUAAAC
1125	CCCAGCU C CUGCUGA	1565	UACCUU A UAACCGC
1163	CGCAGCU U CUUCUGC	1567	CCUCUUA A ACCGCCA
1164	GCAGCUU C UCCUGCU	1584	GGAAGAU C AAGAAAU
1166	AGCUUCU C CUGUCUU	1592	AAGAAU A CAGACUA
1172	UCCUGCU C UGCAACC	1599	ACAGACU A CAACAGG
1200	GCCAGCU U AUACACA	1651	CACGCCU C CCUGAAC
1201	CCAGCUU A UACACAA	1661	UGAACCU A UCCCGGG
1203	AGCUUAU A CACAAGA	1663	AACCUUA C CCGGGAC
1227	GGGAGCU U CGUGUCC	1678	AGGGCCU C UUCUUG
1228	GGAGCUU C GUGUCCU	1680	GGCCUUC U CCUGGCG
1233	UUCGUGU C CUGUAUG	1681	GCCUUCU C CUCGGCC
1238	GUCCUGU A UGGCCCC	1684	UCUUCU C GGCUUUC
1264	GAGGGAU U GUCCGGG	1690	UCGGCCU U CCCAUUA
1267	GGAUUGU C CGGGAUA	1691	CGGCCUU C CCUAUUA
1294	AGAAAUU U CCCAGCA	1696	UUCUCAU A UUGGUGG
1295	GAAAUUU C CCAGCAG	1698	CCCAUUA U GGUGGCA
1306	GCAGACU C CAAGUGU	1737	AAGACAU A UGCCAUG
1321	CCAGGCU U GGGGGAA	1750	UGCAGCU A CACCUAC
1334	AACCCAU U GCGCGAG	1756	UACACCU A CCGGCC
1344	CCGAGCU C AAGUGUC	1787	AGGGCAU U GUCCUCA
1351	CAAGUGU C UAAAGGA	1790	GCAUUGU C CUCAGUC
1353	AGUGUCU A AAGGAUG	1793	UUGUCCU C AGUCAGA
1366	UGGCACU U UCCACU	1797	CCUCAGU C AGAUACA
1367	GGCACUU U CCCACUG	1802	GUCAGAU A CAACAGC
1368	GCACUUU C CCACUGC	1812	ACAGCAU U UGGGGCC
1380	UGCCCAU C GGGGAUU	1813	CAGCAUU U GGGGCCA
1388	GGGGAUU C AGUGACU	1825	CCAUUGU A CCUGCAC
1398	UGACUGU C ACUCGAG	1837	CACACCU A AAACACU
1402	UGUCACU C GAGAUUU	1845	AAACACU A GGCCACG

1856	CACGCAU C UGADCUG	2189	UAUUUAU U GAGUGUC
1861	AUCUGAU C UGUAGUC	2196	UGAGUGU C UUUUAUG
1865	GADCUGU A GUCACAU	2198	AGUGUCU U UUAUGUA
1868	CUGUAGU C ACAUGAC	2199	GUGUCUU U UADGUAG
1877	CAUGACT A AGCCAAG	2200	UGUCUUU U AUGUAGG
1901	CAAGACU C AAGACAU	2201	GUCUUUU A UGUAGGC
1912	ACAUGAU U GAUGGAU	2205	UUUAUGU A GGUAAA
1922	UGGAUGU U AAAGUCU	2210	GUAGGCU A AAUGAAC
1923	GGAUUU A AAGUCUA	2220	UGAACAU A GGUUCU
1928	UUAAGU C UAGCCUG	2224	CAUAGGU C UCGGCC
1930	AAAGUCU A GCCUGAU	2226	UAGGUCU C UGGCCUC
1964	GAGACAU A GCCCCAC	2233	CUGGCCU C ACGGAGC
1983	AGGACAU A CAACUGG	2242	CGGAGCU C CCAGUCC
1996	GGGAAAU A CUGAAAC	2248	UCCAGU C CAUGUCA
2005	UGAAACU U GCUGCCU	2254	UCCADGU C ACADUCA
2013	GCUGCCU A UUGGGUA	2259	GUACAUU U CAAGGUC
2015	UGCCUAU U GGGUADG	2260	UCACADU C AAGGUCA
2020	AUUGGGU A UGCUGAG	2266	UCAAGGU C ACCAGGU
2039	ACAGACU U ACAGAAG	2274	ACCAGGU A CAGUGU
2040	CAGACUU A CAGAAGA	2279	GUACAGU U GUACAGG
2057	UGGCCCU C CAUAGAC	2282	CAGUUGU A CAGGUUG
2061	CCUCCAU A GCAUGU	2288	UACAGGU U GUACACU
2071	CAUGUGU A GCAUCAA	2291	AGGUUGU A CACUGCA
2076	GUAGCAU C AAAACA	2321	AAAAGAU C AAAUGGG
2097	CCACACU U CCUGACG	2338	UGGGACU U CUCAUUG
2098	CACACUU C CUGACGG	2339	GGGACUU C UCADUGG
2115	GCCAGCU U GGGCACU	2341	GACUUCU C AUUGGCC
2128	CUGCUGU C UACUGAC	2344	UUUCUAU U GGCCAC
2130	GCUGUCU A CUGACCC	2358	CCUGCCU U UCCCCAG
2145	CAACCCU U GAUGAUA	2359	CUGCCUU U CCCAGA
2152	UGAUGAU A UGUUUU	2360	UGCCUUU C CCCAGAA
2156	GAUAUGU A UUAUUC	2376	GAGUGAU U UUUCUAU
2158	UAUGUAU U UAUUCAU	2377	AGUGAUU U UUCUAUC
2159	AUGUAUU U AUUCAUU	2378	GUGAUUU U UCUAUCG
2160	UGUAUUU A UUCAUUU	2379	UGAUUUU U CUADCGG
2162	UAUUUAU U CAUUUGU	2380	GAUUUUU C UADCGGC
2163	AUUUAUU C AUUUGUU	2382	UUUUUCU A UCGGCAC
2166	UAUUCAU U UGUUAUU	2384	UUUCUAU C GGCACAA
2167	AUUCAUU U GUUAUUU	2399	AAGCACU A UAUGGAC
2170	CAUUUGU U AUUUUAC	2401	GCACUAU A UGGACTG
2171	AUUUGUU A UUUUACC	2411	GACUGGU A AUGGUUC
2173	UUGUUAU U UUAACCAG	2417	UAUUGGU U CACAGGU
2174	UGUUAUU U UACCAGC	2418	AAUGGUU C ACAGGUU
2175	GUUAUUU U ACCAGCU	2425	CACAGGU U CAGAGAU
2176	UUUAUUU A CCAGCUA	2426	ACAGGUU C AGAGAUU
2183	ACCAGCU A UUUUUG	2433	CAGAGAU U ACCCAGU
2185	CAGCUAU U UAUUGAG	2434	AGAGAUU A CCCAGUG
2186	AGCUAUU U AUUGAGU	2448	GAGGCCU U AUUCCUC
2187	GCUAUUU A UUGAGUG	2449	AGGCCUU A UUCCUCC

2451	GCCUUAU U CCUCCCU	2750	UAUGUGU A GACAAGC
2452	CCUUAUU C CUCCCUU	2759	ACAAGCU C UCGCUCU
2455	UAUUCUU C CCUCCCC	2761	AAGCUCU C GCUCUGU
2459	CCUCCCU U CCCCCCA	2765	UCUCGCU C UGUCACC
2460	CUCCCUU C CCCCCAA	2769	GCUCUGU C ACCCAGG
2479	GACACCU U UGUUAGC	2797	GUGCAAU C AUGGUUC
2480	ACACCUU U GUUAGCC	2803	UCADGGU U CACUGCA
2483	CCUUUGU U AGCCACC	2804	CAUGGUU C ACUGCAG
2484	CUUUGUU A GCCACCU	2813	CUGCAGU C UUGACCU
2492	GCCACCU C CCCACCC	2815	GCAGUCU U GACCUUU
2504	CCCACAU A CAUUUCU	2821	UUGACCU U UUGGGCU
2508	CAUACAU U UCUGCCA	2822	UGACCUU U UGGGCUU
2509	AUACAUU U CUGCCAG	2823	GACCUUU U GGGCUCA
2510	UACAUUU C UGCCAGU	2829	UUGGGCU C AAGUGAU
2520	CCAGUGU U CACAUG	2837	AAGUGAU C CUCCCAC
2521	CAGUGUU C ACAAUGA	2840	UGAUCCU C CCACUUC
2533	UGACACU C AGGGGUC	2847	CCCACCU C AGCCUCC
2540	CAGCGGU C AUGUCUG	2853	UCAGCCU C CUGAGUA
2545	GUCAUGU C UGGACAU	2860	CCUGAGU A CUGGGA
2568	AGGGAU A UGCCCCA	2872	GGACCAU A GGCUCAC
2579	CCAAGCU A UGCCUUG	2877	AUAGGCU C ACAACAC
2585	UAUGCCU U GUCCUCU	2899	GGCAAAU U UGAUUUU
2588	GCCUUGU C CUUUGU	2900	GCAAAU U GAUUUUU
2591	UUGUCCU C UUGUCCU	2904	AUUUGAU U UUUUUUU
2593	GUCCUCU U GUCCUGU	2905	UUUGAUU U UUUUUUU
2596	CUUUGU C CUGUUUG	2906	UUGAUUU U UUUUUUU
2601	GUCCUGU U UGCAUUU	2907	UGAUUUU U UUUUUUU
2602	UCCUGUU U GCAUUUC	2908	GAUUUUU U UUUUUUU
2607	UUUGCAU U UCACUGG	2909	AUUUUUU U UUUUUUU
2608	UUGCAUU U CACUGGG	2910	UUUUUUU U UUUUUUU
2609	UGCAUUU C ACUGGGA	2911	UUUUUUU U UUUUUUU
2620	GGGAGCU U GCAUUAU	2912	UUUUUUU U UUUUUUC
2626	UUGCACT A UUGCAGC	2913	UUUUUUU U UUUUUCA
2628	GCAUUAU U GCAGCUC	2914	UUUUUUU U UUUCAG
2635	UGCAGCU C CAGUUUC	2915	UUUUUUU U UUUCAGA
2640	CUCCAGU U UCCUGCA	2916	UUUUUUU U UUCAGAG
2641	UCCAGUU U CCUGCAG	2917	UUUUUUU U UCAGAGA
2642	CCAGUUU C CUGCAGU	2918	UUUUUUU U CAGAGAC
2653	CAGUGAU C AGGGUCC	2919	UUUUUUU C AGAGACG
2659	UCAGGGU C CUGCAAG	2931	ACGGGGU C UCGCAAC
2689	CCAAGGU A UUGGAGG	2933	GGGGUCU C GCAACAU
2691	AAGGUAU U GGAGGAC	2941	GCAACAU U GCCCAGA
2700	GAGGACU C CCUCCCA	2951	CCAGACU U CCUUUGU
2704	ACUCCCU C CCAGCUU	2952	CAGACUU C CUUUGUG
2711	CCCAGCU U UGGAAGG	2955	ACUCCCU U UGUGUUA
2712	CCAGCUU U GGAAGGG	2956	CUUCCCU U GUGUAG
2721	GAAGGGU C AUCCGCG	2961	UUUGUGU U AGUUAUU
2724	GGGUCAU C CGCGUGU	2962	UUGUGUU A GUUAUA
2744	UGUGUGU A UGUGUAG	2965	UGUUGU U AAUAAG

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2966	GUUAGUU	A	AUAAAGC
2969	AGUUAAU	A	AAGCUUU
2975	UAAAGCU	U	UCUCAAC
2976	AAAGCUU	U	CUCAACU
2977	AAGCUUU	C	UCAACUG
2979	GCUUUCU	C	AACUGCC

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Table 3

Mouse ICAM HH Target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
11	CCUGGU C acCGUG	367	AAUGGU u cAAACCg
23	CaGuGgU u CUCUGCU	374	gAAGCCU U CCUGcCC
26	uGgUuCU C UGUUcCU	375	AAgCCU C CUgcCCc
31	CUCUGCU c CUCcaca	378	CuacCaU C ACCGUGU
34	UuCUcaU a AGgGUcG	386	ACCGUGU A uUcGuuU
40	gCacAcU U GuAgCCU	394	CcGGACU u ucGAuCu
48	aggACCU C AGCCUgG	420	CACaCuU C CCCcCcg
54	UggGCCU C GuG AUGG	425	CaCCCCU C ccaGCAG
58	CaUgcCU u UaGCUCC	427	CagCCU c aGCAgug
64	cAcccCU C CCAGCAG	450	AGgACCU c ACCCUgC
96	CucugCU C CUGGcCC	451	GAAaCcU u uCCUuuG
102	UgCcaGU a CUGCUgG	456	UUAACCU c aGCcaCu
108	cuCUGCU C cuGGCcC	495	CuAcCaU C ACCGUGu
115	uGGuuCU C UGcUCCu	510	UGCUGCU C CGUGGGG
119	GgaaUGU c aCCAGGA	564	CUcAGGU a uCcAuCc
120	CUCUGcU C CUGGccC	592	GAaAGAU C ACaugGG
146	CAGuGgU C cGcuUCC	607	AGCCAAU U UCDCaUG
152	UCUGUGU C agCCAaCu	608	GCCAAU U CUcaUGC
158	UCCugU u AAAAacC	609	CCAAUU C UCauGCC
165	CagAAGU u gUuuUGC	611	AAUUUCU C aUGCCGC
168	AAGcCuU C CUGCCCC	656	aAGCUGU U UGAGcug
185	GGUGGgU C CGUGCaG	657	AGCUGUU U GAGcugA
209	gcCACuU C CUcUGgC	668	cgagCCU a GGCCaCC
227	CagAAGU U GUUuuGC	677	GaCCuCU A CCAGCcu
230	AAGUUGU U uuGCucc	684	uuCAGCU C CgGuCCU
237	UGuGCuU u GAGAaCu	692	CgGACuU U cGauCUu
248	AaCCCaU c uCCUAAA	693	AGgaCcU c acCCUGC
253	ccUGCCU A AggAaGA	696	CCUgUuU C CUGCCuc
263	AgGGuuU c uCUaCUG	709	gGCGgCU C CaCCuCA
267	AGggGCU C CUGCCUa	720	uACAACTU U uUCAGCu
293	AAGcUGU u UGAgCUG	723	AACUUuU C AGCuCCg
319	AGgAGAU A cugAgCC	735	aCCaGaU C CUgGAGa
335	cUGUGCU u UygAAC	738	uGGgCCU c GuGaUGG
337	GUcCaAU U CACACUG	765	CaGUcGU C cGcUuCC
338	aGCUgUU u gAgCUGa	769	GGcCUGU U uCCUGcc
359	GuGCAGU C guCcGCU	770	uUuUGcU C CUGGAa
785	GGcCUGU U uCCuGcC	1353	AGUGggU c gAaGgUG
786	GcCUGUU u CCuGcCU	1366	UaaCAGU c UaCaACU
792	UggagGU C UCGGAaG	1367	aGCACcU c CCCACcu
794	CugGgCU u GGAGaCu	1368	GuACUgU a CCACUcu
807	CuGgGaU a uACCUGG	1380	UGCCCAU C GGGGugg
833	CAzAGcU c GacaCCC	1388	GGaGAcU C AGUGgCU
846	CCcugGU C ACCguUG	1398	UGgCUGU C ACagaAc
851	GagACCU c UacCAgC	1402	UGUgcU u GAGAaCU

863	AgCcACU u CcUCUG	1408	gCGAGAU C ggGgaGG
866	GAagCCU U CcuGcCC	1410	GAGgUCU c GgaaGgg
867	AuUCgUU u cCGGagA	1421	ccCACCU A CuUuUGU
869	UCuUcCU C augCAAG	1425	aCUgCCU u gGUaGaG
881	AuGGCuU C AacCcGU	1429	uCUcUaU u GccCCuG
885	CCUugGU a gagGUGA	1444	GAaggCU C AgGaGGA
933	cUauAaU c ADuCUGG	1455	GGaAuGU C ACCaGga
936	uAaUcAU u CUGGuGc	1482	AguUGuU u UgCuCCC
978	UaACagU C UACAaCU	1484	cUGuUCU u CCuCauG
980	ACagUCU A CAaCUUU	1493	CuguGcU u UGAGAac
986	UACAaCU U UuCaGcu	1500	ADGAaAU c aUggUCc
987	ACAaCUU U uCaGcuC	1503	gGAcUaU a ADCADuc
988	CAaCUUU u CaGcuCC	1506	UUaUguU u AUaACcG
1005	ACcaGAU c CUGgaGA	1509	cuAcCAU C ACCGUGu
1006	uGaGAgU C UGggGAA	1518	ucaUGGU c cCAGgCG
1023	ugGAGGU C UGgGAAG	1530	CuauAaU C AUucUGG
1025	GAGGUcU c gGAAGGG	1533	ugGUCAU u gUGGGCc
1066	CCAuCU c aAaauAA	1551	CAuGCCU u AGCagcU
1092	AcuGGAU c uCAGgCC	1559	AGCACcU c CCcaccU
1093	UGGaccU u CAGCCAa	1563	CuUAuGU u UAUAAcc
1125	CCCAaCU c uUcuUGA	1565	UAugUuU A UAACCGC
1163	CGaAGCU U CUuuUGC	1567	ugUuUAU A ACCGCCA
1164	GaAGCUU C UuuUGCU	1584	GaAAGAU C AgGAuAU
1166	AGCUUCU u uUGCUCU	1592	AgGAuAU A CAaguUA
1172	UCCUGuU u aaaAACC	1599	ACAaguU A CAgaAGG
1200	cuCuGCU c cUcCACA	1651	CcCaCCU C CCUGAgC
1201	gCuGCUU u UgaACAg	1661	gaAACCU u UCCuuuG
1203	AcuUuuU u CACcAGu	1663	AACCUuU C CuuuGAA
1227	GGuAcaU a CGUGUGC	1678	AGGaCCU C agCCUGg
1228	GaAGCUU C uUuUGCU	1680	aGCCaCU U CCUCuGg
1233	UUCGUuU C CgGagaG	1681	GCCaCUU C CUcuGgC
1238	GUgCUGU A UGUcCCu	1684	aCUUCCU C uGgCUgu
1264	GAaGGgU c GUgCaaG	1690	cCGGaCU U uCgAUcU
1267	uGAgAGU C uGGGgAA	1691	CGGaCUU u CgAUcUU
1294	AGgAgAU a CugAGCc	1696	UgCCCAU c ggGGUGG
1295	GAggggU C uCAGCAG	1698	CggAUUA a ccUGGag
1306	GCAGACU C ugAaaUG	1737	gAGACcU c UaCCAgc
1321	gaAGGCU c aGGAgGA	1750	gGCgGCU c CACCUca
1334	AACCCAU c uCCuaAa	1756	gAagCCU u CCuGCCC
1344	auGAGCU C gAGaGUg	1787	gaGaCAU U GUCCcCA
1351	ugAaUGU a UAAguuA	1790	GCAUUGU u CUCuaau
1793	UgGUCCU C gGcugGA	2173	UUagagU U UUAACCAG
1797	CacCAGU C AcAUaAa	2174	UagagUU U UACCAGC
1802	acCAGAU c CuggAGa	2175	agagUUU U ACCAGCU
1812	ACuGgAU c UcaGGCC	2176	gagUUUU A CCAGCUA
1813	CAGCAUU U acccuCA	2183	ACCAGCU A UUUUAUG
1825	CCAcGcU A CCUCugC	2185	CAGCUAU U UAUUGAG
1837	CAugCCU u uAgCuCc	2186	AGCUAUU U AUUGAGU
1845	cgAgcCU A GGCCACC	2187	GCUAUUU A UUGAGUa

1856	CggaCuU u cGADCUu	2189	UAUUUAU U GAGUacC
1861	AcaUGAU a UccAGUa	2196	caAcUcU u cUUgADG
1865	cAcuUGU A GcCuCag	2198	gcaGcCU c UUAUGUu
1868	CaccAGU C ACAUaAa	2199	GccUCCU a UgUuUAu
1877	CAUGcCU u AGCagcu	2200	UcUuccU c ADGcAaG
1901	uAAaACU C AAGggAc	2201	aagUUUU A UGUcGGC
1912	AuAUagU a GADcagU	2205	UUUAUGU c GGcugA
1922	UGaAUGU a uAAGUua	2210	GgAGaCU c AgUGgcu
1923	uGAUGcU c AgGUaUc	2220	cuggCAU u GuUCCU
1928	UUAgAGU u UuaCCaG	2224	CucAGGU a UCcauCC
1930	AgAGUuU u aCCaGcU	2226	UgGaUcU C aGGCCGc
1964	GAGACAU u GuCCCCa	2233	CUGaCCU C cuGGAGg
1983	AGGauAU A CAAGUua	2242	uGGAGCU a gCgGACC
1996	aGGAgAU A CUGAgcC	2248	UauCcaU C CAUccCA
2005	UGgAgCU a GCgGaCc	2254	UCCAauU C ACAcUGA
2013	GCUauuU A UUGaGUA	2259	aUCACAU U CAcGGUg
2015	UGCCcAU c GGGgugG	2260	UCACAUU C AcGGUgc
2020	ggUGGuU c UuCUGAG	2266	ggAAuGU C ACCAGGa
2039	gCuGgCU a gCAGAgG	2274	ACCAGaU c CuGgaGa
2040	CuGACcU c CuGgAGg	2279	GaAggGU c GUgCAaG
2057	UGcuCCU C CAcAucc	2282	aAGcUGU u ugaGcUG
2061	CuaCCAU c acCgUGU	2288	UAAaAGU U aUggcCU
2071	CACuUGU A GCcUCCag	2291	caGUgGU u CuCUGCu
2076	GUAGCcU C AgAgCua	2321	gAAAGAU C AcAUGGG
2097	CaACuCU U CuUGauG	2338	UGaGACU c CUgcccUG
2098	CACACUU C CccccCcG	2339	GaaACcU u UCcUUUg
2115	GCCAGCU c GGaggau	2341	GACcUcU a ccaGcCu
2128	CaGCUaU u UAuUGAg	2344	UUucgAU c uuCCAgC
2130	cCUGUuU c CUGcCuC	2358	CCcagCU c UCagCAG
2145	CAACuCU U cuUGAUg	2359	CUGCuUU U gaaCAGA
2152	UauUaAU u UagAgUU	2360	aaCCUUU C CUuuGAA
2156	uugAUGU A UUUUAUa	2376	agGUGgU U cUUUcga
2158	gAUGUAU U UAUUaAU	2377	gGUGgUU c UUCUgag
2159	AUGUAUU U AUUaAUU	2378	agGgUUU c UCUAcuG
2160	UGUAUUU A UUAaUUU	2379	UGcUUUU c ucAUaaG
2162	UAUUUAU U aAUUUag	2380	aAgUUUU a UgUCCGC
2163	AUGUAUU u AUUaaUU	2382	aUUcUcU A UuGcCcC
2166	acUUCAU U cucUAUU	2384	aUcCagU a GaCACAA
2167	AUguAUU U aUUaaUU	2399	AAaCACU A UgUGGAC
2170	uAUUUaU U AaUUUAg	2401	aagCUgU u UGagCUG
2171	AgUUGUU u UgCUCcC	2411	uACUGGU c AgGaUgC
2417	gAAUGGU a CAuAcGU	2691	AAuGUcU c cCAGGcC
2418	AcUGGaU C uCAGGcc	2700	GAaGcCU u CCUgCCc
2425	CAugGGU c gAGgGuU	2704	gacCuCU a CCAGCcU
2426	AuuaaUU u AGAGuUU	2711	CCCAGCU c UcagcaG
2433	uAGAGuU U uaCCAGc	2712	gagGucU c GGAAGGG
2434	AGAGuUU u aCCAGcu	2721	GAAGGGU c gUgCaaG
2448	GAaGCCU U ccUgCcC	2724	GGuaCAU a CGuGUGc
2449	AaGCCUU c cUgCcCC	2744	gGUGgGU c cGUGcAG

2451	GCCUguU U CCUGCCU	2750	UAUuUaU u GAguAcC
2452	CCUguUU C CUgCCUc	2759	cCggaCU u UCGaUCU
2455	gAagCCU u CCUGCCC	2761	AgGacCU C aCcCUGc
2459	CCaCaCU U CCCCCCc	2765	UuUuGCU C UGcCgCu
2460	CaCaCUU C CCCCCcg	2769	agUcUGU C AaaCAGG
2479	GAgACCU c UaccAGC	2797	aUGaAAU C AUGGUcC
2480	uCACCGU U GUgAuCC	2803	UCAUGGU c CcagGCg
2483	CCaaUGU c AGCCACC	2804	ggUGGGU C cgUGCAG
2484	CUUUhUU c aCCAguc	2813	CUcCgGU C cUGACCc
2492	agCACCU C CCCACCU	2815	aCAGUCU a cAaCUUU
2504	CCCACcU A CuUUUGU	2821	cUGACCU c cUGGagg
2508	uAUcCAU c caUcCCA	2822	gGAgCcu c cGGaCUu
2509	uUAgAgU U uUaCCAG	2823	ugCCUUU a GcuCcCA
2510	UAgAgUU u UaCCAGc	2829	cUGGaCU a uAaUcAU
2520	CuuuUGU U CcCA AUG	2837	AgGUGgU u CUuCuGa
2521	CAGcaUU u ACccUcA	2840	UGAgACU C CugCCUg
2533	UGAugCU C AGguaUC	2847	CCaAugU C AGCCaCC
2540	CAGCaGU C cgcUgUG	2853	gCAGCCU C uUauGUu
2545	GUgcUGU a UGGuCcU	2860	gCcaAGU A aCUGuGA
2568	guGaAgU c UGuCaAA	2872	GGACCUU c aGCCaAg
2579	auAAGuU A UGgCcUG	2877	uUccGCU a cCAuCAC
2585	cugGCaU U GUuCUU	2899	cGgAcuU U cGAdCUU
2588	GCaUUGU u CUUaaU	2900	uuAAuUU a GAgUUUU
2591	UGGUuCU C UgcUCCU	2904	AcUUcAU U cUcUaUU
2593	cUuCUuU U GcuUGc	2905	cUUCADU c UcUaUUG
2596	CUuUUGU u CccaaUG	2906	UUGAUgU a UUuaUUa
2601	acCgUGU a UuCgUUU	2907	UGuaUUU a UUaaUUU
2602	UCCaGcU a cCAUccC	2908	GAagcUU c UUUUgcU
2607	cUcGgAU a UacCUGG	2909	AgcUUcU U UUGcUcU
2608	caGCAgU c CgCUGuG	2910	UgUaUUU a UUaaUUU
2609	gGaAUgU C ACcaGGA	2911	UgUaUUU a UUaaUUU
2620	aGGAcCU c aCcUgc	2912	UUgUUcU c UaaUgUC
2626	UUuCGaU c UUCcAGC	2913	UUUcUcU a cUggUCA
2628	GCAcAcU U GuAGccu	2914	UgcUUUU c UcaUaAG
2635	UuCAGCU C CgGUccu	2915	aUUUaUU a aUUuAGA
2640	ggCCuGU U UCCUGCc	2916	UaUUcgU U UcCgGAG
2641	cCCAGcU c uCaGCAG	2917	aUUcgUU U cCgGAGA
2642	CCuGUUU C CUGCcuC	2918	UUcgUUU c CgGAGAg
2653	uAcUGgU C AGGaUgC	2919	UUcUcaU a AGgGuCG
2659	gaAGGGU C* gUGCAAG	2931	ugGaGGU C UCGGAAG
2689	CuAAuGU c UccGAGG	2933	GaGGUCU C GgAAagg
2941	GagACAU U GuCCccA		
2951	CCAcgCU a CCUcUGc		
2952	CAGcagU C CgcUGUG		
2955	AgUGaCU c UGUGUcA		
2956	uUUCCUU U GaaUcAa		
2961	UcUGUGU c AGccAcU		
2962	aUGUaUU u aUUAAUu		
2965	UuUgAaU c AAUAAAG		

2966	GcUgGcU	A	gcAgAGg
2969	aaUcAAU	A	AAGuUUU
2975	UAgAGuU	U	UacCAgC
2976	gAgGgUU	U	CUcuACU
2977	AAGCUgU	u	UgAgCUG
2979	uCaUUCU	C	uAuUGCC

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Table 4
Human ICAM HH Ribozyme Sequences

nt. Position	Ribozyme Sequence
11	CAGCGUC CUGAUGAGGCCGAAAGGCCGAA ACUGGGG
23	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCUCAG
26	AGUAGCA CUGAUGAGGCCGAAAGGCCGAA AGGAGCU
31	CTCUGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
34	CAACUCU CUGAUGAGGCCGAAAGGCCGAA AGUAGCA
40	AGGUUGC CUGAUGAGGCCGAAAGGCCGAA ACUCUGA
48	CGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUUGC
54	CCAUAGC CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
58	GGAGCCA CUGAUGAGGCCGAAAGGCCGAA AGCGAGG
64	CDGCUGG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
96	GGACCAG CUGAUGAGGCCGAAAGGCCGAA AGUGCGG
102	CGAGCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGA
108	GAGCCOC CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
115	GGGAACA CUGAUGAGGCCGAAAGGCCGAA AGCCCCG
119	UCCUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGAGC
120	GUCCUGG CUGAUGAGGCCGAAAGGCCGAA AACAGAG
146	GGACACA CUGAUGAGGCCGAAAGGCCGAA AUGUCUG
152	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA ACACAGA
158	GACUUUU CUGAUGAGGCCGAAAGGCCGAA AGGGGGA
165	GCAGGAU CUGAUGAGGCCGAAAGGCCGAA ACUUUUG
168	GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AUGACUU
185	CAGCACG CUGAUGAGGCCGAAAGGCCGAA AGCCUCC
209	GUCACAG CUGAUGAGGCCGAAAGGCCGAA AGGUGCU
227	GCCCCAC CUGAUGAGGCCGAAAGGCCGAA ACTUGGG
230	U AUGCCC CUGAUGAGGCCGAAAGGCCGAA ACAACUU
237	GGGUCUC CUGAUGAGGCCGAAAGGCCGAA AUGCCCA
248	UUUAGGC CUGAUGAGGCCGAAAGGCCGAA ACGGGGU
253	UCCUUUU CUGAUGAGGCCGAAAGGCCGAA AGGCAAC
263	CAGGAGC CUGAUGAGGCCGAAAGGCCGAA ACTUCCU
267	CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAACU
293	CAGUUCA CUGAUGAGGCCGAAAGGCCGAA ACACCUU
319	GGUUGGC CUGAUGAGGCCGAAAGGCCGAA AUUCUUC
335	GUUUGAA CUGAUGAGGCCGAAAGGCCGAA AGCACAU
337	CAGUUUG CUGAUGAGGCCGAAAGGCCGAA AUAGCAC
338	GCAGUUU CUGAUGAGGCCGAAAGGCCGAA AAUAGCA
359	AGCUGUU CUGAUGAGGCCGAAAGGCCGAA ACTUGCC
367	AAGGUUU CUGAUGAGGCCGAAAGGCCGAA AGCUGUU
374	GGUGAGG CUGAUGAGGCCGAAAGGCCGAA AGGUUUU
375	CGGUGAG CUGAUGAGGCCGAAAGGCCGAA AAGGUUU
378	ACACGGU CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
386	AGUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACGGU
394	CGUUCUG CUGAUGAGGCCGAAAGGCCGAA AGUCCAG
420	AAGAGGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUG
425	CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AGGGGAG

427	GGCUGCC	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGG
450	GUAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCU
451	CGUAGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUUC
456	GGCAGCG	CUGAUGAGGCCGAAAGGCCGAA	AGGGUAA
495	CCACGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGG
510	CCCCACG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
564	UGGUCCU	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCG
592	CCAUGGU	CUGAUGAGGCCGAAAGGCCGAA	AUCUCUC
607	CAOGAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCU
608	GCACGAG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGC
609	GGCACGA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGG
611	GCGGCAC	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUU
656	GUUCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUC
657	UGUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AACAGCU
668	GGGGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUU
677	GAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGGC
684	AGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGU
692	CAGGACA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUG
693	GCAGGAC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCU
696	CUGGCAG	CUGAUGAGGCCGAAAGGCCGAA	ACAAAGG
709	UGUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUCCGU
720	GGCUGAC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUG
723	GGGGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAAGUU
735	CCUCUAG	CUGAUGAGGCCGAAAGGCCGAA	ACCCGGG
738	CCACCUC	CUGAUGAGGCCGAAAGGCCGAA	AGGACCC
765	GGGAACA	CUGAUGAGGCCGAAAGGCCGAA	ACCACGG
769	UCCAGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGACC
770	GUCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGAC
785	GACUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGCCC
786	AGACUGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGCC
792	CCUCCGA	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGA
794	GGCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACUGG
807	CCAGGUG	CUGAUGAGGCCGAAAGGCCGAA	ACCUGGG
833	GGGGUUC	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCG
846	CAUAGGU	CUGAUGAGGCCGAAAGGCCGAA	ACUGUGG
851	GUUGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGGUGAC
863	CGAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCGUU
866	GGCCGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGUC
867	UGGCCGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAGU
869	CUUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGAAGGA
881	ACUGACU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUU
885	UCACACU	CUGAUGAGGCCGAAAGGCCGAA	ACUGAGG
933	CCAGUAU	CUGAUGAGGCCGAAAGGCCGAA	ACUGCAC
936	UCCCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUUAACU
978	AGCUGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGUCA
980	AAAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGU
986	CGCCGGA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGUA
987	GCGCCGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGU
988	GGCGCCG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCUG

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NUC 37788

1005	UCGUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCACGU
1006	UUCGUCA	CUGAUGAGGCCGAAAGGCCGAA	AAUCACG
1023	CUUCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUUCG
1025	CCCUCUC	CUGAUGAGGCCGAAAGGCCGAA	AGACCUUC
1066	UUGGCUC	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUG
1092	GGGCUGG	CUGAUGAGGCCGAAAGGCCGAA	ACCCCAU
1093	UGGGCUG	CUGAUGAGGCCGAAAGGCCGAA	AACCCCA
1125	UCAGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
1163	GCAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCG
1164	AGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGC
1166	AGAGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCU
1172	GGUUGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGA
1200	UGUGUAU	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGC
1201	UUGUGUA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGG
1203	UCUUGUG	CUGAUGAGGCCGAAAGGCCGAA	AUAAGCU
1227	GGACACG	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCC
1228	AGGACAC	CUGAUGAGGCCGAAAGGCCGAA	AAGCUC
1233	CAUACAG	CUGAUGAGGCCGAAAGGCCGAA	ACAACGA
1238	GGGGCCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGAC
1264	CCCGGAC	CUGAUGAGGCCGAAAGGCCGAA	AUCCUCUC
1267	UUUCCCG	CUGAUGAGGCCGAAAGGCCGAA	ACAAUCC
1294	UGCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUC
1295	CUCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AADUUUC
1306	CACAUUG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUGC
1321	UUCCCCC	CUGAUGAGGCCGAAAGGCCGAA	AGCCUGG
1334	CUCGGGC	CUGAUGAGGCCGAAAGGCCGAA	AUGGGUU
1344	GACACTU	CUGAUGAGGCCGAAAGGCCGAA	AGCUCGG
1351	UCCUUUA	CUGAUGAGGCCGAAAGGCCGAA	ACACUUG
1353	CAUCCUU	CUGAUGAGGCCGAAAGGCCGAA	AGACACT
1366	AGUGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGUGCCA
1367	CAGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGCC
1368	GCAGUGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUGC
1380	AUCCCCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
1388	AGUCACU	CUGAUGAGGCCGAAAGGCCGAA	AUUCCCC
1398	CUCGAGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGUCA
1402	AGAUCUC	CUGAUGAGGCCGAAAGGCCGAA	AGUGACA
1408	CCCUCAA	CUGAUGAGGCCGAAAGGCCGAA	AUCUCGA
1410	UGCCUCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCU
1421	ACAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGCC
1425	CCCGACA	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGG
1429	CUGGCCC	CUGAUGAGGCCGAAAGGCCGAA	ACAGAGG
1444	UCCCCUU	CUGAUGAGGCCGAAAGGCCGAA	AGUGCUC
1455	CGCGGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCUC
1482	GGGGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGCACAU
1484	CCGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGCAC
1493	AAUCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACCGGGG
1500	UGAUGAC	CUGAUGAGGCCGAAAGGCCGAA	AUCUCAU
1503	UGAUGAU	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUC
1506	CAGUGAU	CUGAUGAGGCCGAAAGGCCGAA	AUGACAA

1509	CCACAGU	CUGAUGAGGCCGAAAGGCCGAA	AUGADGA
1518	CGGCUGC	CUGAUGAGGCCGAAAGGCCGAA	ACCACAG
1530	CCAUUAU	CUGAUGAGGCCGAAAGGCCGAA	ACUGCGG
1533	UGCCCAU	CUGAUGAGGCCGAAAGGCCGAA	AUGACUG
1551	ACGUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUG
1559	AUAGAGG	CUGAUGAGGCCGAAAGGCCGAA	ACGUGCU
1563	GGUUAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGUACG
1565	GCGGUUA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUA
1567	UGGCGGU	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGG
1584	AUUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUCU
1592	UAGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUU
1599	CCUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUGU
1651	GUUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCGUG
1661	CCCGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCA
1663	GUCCCGG	CUGAUGAGGCCGAAAGGCCGAA	AUAGGUU
1678	CGAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCU
1680	GCCGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGCC
1681	GGCCGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAGGC
1684	GAAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGA
1690	AUAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGA
1691	AUAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCG
1696	CCACCAA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGAA
1698	UGCCACC	CUGAUGAGGCCGAAAGGCCGAA	AUAUGGG
1737	CAUGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUU
1750	GUAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCA
1756	GGGCCGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUA
1787	UGAGGAC	CUGAUGAGGCCGAAAGGCCGAA	AUGCCCU
1790	GACUGAG	CUGAUGAGGCCGAAAGGCCGAA	ACAAUGC
1793	UCUGACU	CUGAUGAGGCCGAAAGGCCGAA	AGGACAA
1797	UGUAUCU	CUGAUGAGGCCGAAAGGCCGAA	ACUGAGG
1802	GCUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAC
1812	GGCCCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCUGU
1813	UGGCCCC	CUGAUGAGGCCGAAAGGCCGAA	AAUGCTG
1825	GUGCAGG	CUGAUGAGGCCGAAAGGCCGAA	ACCAUGG
1837	AGUGUUU	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUG
1845	CGUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUU
1856	CAGAUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCGUG
1861	GACUACA	CUGAUGAGGCCGAAAGGCCGAA	AUCAGAU
1865	AUGUGAC	CUGAUGAGGCCGAAAGGCCGAA	ACAGAUU
1868	GUCAUGU	CUGAUGAGGCCGAAAGGCCGAA	ACUACAG
1877	CUUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AGUCAUG
1901	AUGUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUUG
1912	AUCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AUCAUGU
1922	AGACUUU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCCA
1923	UAGACUU	CUGAUGAGGCCGAAAGGCCGAA	AACAUCC
1928	CAGGCUA	CUGAUGAGGCCGAAAGGCCGAA	ACUUUAA
1930	AUCAGGC	CUGAUGAGGCCGAAAGGCCGAA	AGACUUU
1964	GUGGGGC	CUGAUGAGGCCGAAAGGCCGAA	AUGUCTU
1983	CCAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUCCU

1996	GUUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCCC
2005	AGGCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCA
2013	UACCCAA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGC
2015	CAUACCC	CUGAUGAGGCCGAAAGGCCGAA	AUAGGCA
2020	CUCAGCA	CUGAUGAGGCCGAAAGGCCGAA	ACCCAAU
2039	CUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUGU
2040	UCUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUG
2057	GUCUADG	CUGAUGAGGCCGAAAGGCCGAA	AGGGCCA
2061	ACAUGUC	CUGAUGAGGCCGAAAGGCCGAA	AUGGAGG
2071	UUGAUGC	CUGAUGAGGCCGAAAGGCCGAA	ACACAUG
2076	GUGUUUU	CUGAUGAGGCCGAAAGGCCGAA	AUGCUAU
2097	CGUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGUGG
2098	COGUCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUG
2115	AGUGCCC	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGC
2128	GUCAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAG
2130	GGGUCAG	CUGAUGAGGCCGAAAGGCCGAA	AGACAGC
2145	UAUCAUC	CUGAUGAGGCCGAAAGGCCGAA	AGGGUUG
2152	AAAUACA	CUGAUGAGGCCGAAAGGCCGAA	AUCAUCA
2156	GAADAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUC
2158	AUGAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUACAUU
2159	AAUGAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2160	AAAUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2162	ACAAAUG	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
2163	AACAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
2166	AAUAACA	CUGAUGAGGCCGAAAGGCCGAA	AUGAAUA
2167	AAAUAAC	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAU
2170	GUA AAAU	CUGAUGAGGCCGAAAGGCCGAA	ACAAAUG
2171	GGUAAAA	CUGAUGAGGCCGAAAGGCCGAA	AACAAAU
2173	CUGGUAA	CUGAUGAGGCCGAAAGGCCGAA	AUAACAA
2174	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AAUAACA
2175	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAC
2176	UAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAAUAUA
2183	CAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGU
2185	CUCAUAU	CUGAUGAGGCCGAAAGGCCGAA	AUAGCUG
2186	ACUCAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGCU
2187	CACUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGC
2189	GACACUC	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
2196	CAUAAAA	CUGAUGAGGCCGAAAGGCCGAA	ACACUCA
2198	UACAUAA	CUGAUGAGGCCGAAAGGCCGAA	AGACACU
2199	CUACAUU	CUGAUGAGGCCGAAAGGCCGAA	AAGACAC
2200	CCUACAU	CUGAUGAGGCCGAAAGGCCGAA	AAAGACA
2201	GCCUACA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAC
2205	UUUAGCC	CUGAUGAGGCCGAAAGGCCGAA	ACAUAAA
2210	GUUCAUU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUAC
2220	AGAGACC	CUGAUGAGGCCGAAAGGCCGAA	AUGUUCA
2224	GGCCAGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUAUG
2226	GAGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGACCUA
2233	GCUCCGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCAG
2242	GGACUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCG

2248	UGACAUG	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGA
2254	UGAADUGU	CUGAUGAGGCCGAAAGGCCGAA	ACADGGA
2259	GACCUUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUGAC
2260	UGACCUU	CUGAUGAGGCCGAAAGGCCGAA	AADUGA
2266	ACCUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCUGA
2274	ACAACUG	CUGAUGAGGCCGAAAGGCCGAA	ACCUGGU
2279	CCUGUAC	CUGAUGAGGCCGAAAGGCCGAA	ACUGUAC
2282	CAACCUG	CUGAUGAGGCCGAAAGGCCGAA	ACRACUG
2288	AGUGUAC	CUGAUGAGGCCGAAAGGCCGAA	ACCUGUA
2291	UGCAGUG	CUGAUGAGGCCGAAAGGCCGAA	ACAACCU
2321	CCCAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUU
2338	CAUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCCCA
2339	CCAADGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCC
2341	GGCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUC
2344	GUUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AUGAGAA
2358	CUGGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGG
2359	UCUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCAG
2360	UUCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGCA
2376	AUAGAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCACUC
2377	GAUAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAUCACU
2378	CGAUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAADCAC
2379	CCGAUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAADCA
2380	GCCGAUA	CUGAUGAGGCCGAAAGGCCGAA	AAAAADC
2382	GUGCCGA	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAA
2384	UUGUGCC	CUGAUGAGGCCGAAAGGCCGAA	AUAGAAA
2399	GUCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AGUGCUU
2401	CAGUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUAGUGC
2411	GAACCAU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGUC
2417	ACCUGUG	CUGAUGAGGCCGAAAGGCCGAA	ACCAUUA
2418	AACCUUG	CUGAUGAGGCCGAAAGGCCGAA	AACCAUU
2425	AUCUCUG	CUGAUGAGGCCGAAAGGCCGAA	ACCUGUG
2426	AADCUCU	CUGAUGAGGCCGAAAGGCCGAA	AACCUUG
2433	ACUGGGU	CUGAUGAGGCCGAAAGGCCGAA	AUCUCUG
2434	CACUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAUCUCU
2448	GAGGAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUC
2449	GGAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCU
2451	AGGGAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAAGGC
2452	AAGGGAG	CUGAUGAGGCCGAAAGGCCGAA	AADAAGG
2455	GGGAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGAUUA
2459	UGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGAGG
2460	UUGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGGAG
2479	GCUAACA	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUC
2480	GGCUAAC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUGU
2483	GGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAAAGG
2484	AGGUGGC	CUGAUGAGGCCGAAAGGCCGAA	AACAAAG
2492	GGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGC
2504	AGAAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUGGG
2508	UGGCAGA	CUGAUGAGGCCGAAAGGCCGAA	AUGUAUG
2509	CUGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAUGUAU

2510	ACUGGCA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGA
2520	CAUUGUG	CUGAUGAGGCCGAAAGGCCGAA	ACACUGG
2521	UCAUUGU	CUGAUGAGGCCGAAAGGCCGAA	AACACUG
2533	GACCGCU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUCA
2540	CAGACAU	CUGAUGAGGCCGAAAGGCCGAA	ACCGCUG
2545	AUGUCCA	CUGAUGAGGCCGAAAGGCCGAA	ACAUGAC
2568	UUGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUCCCTU
2579	CAAGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUUGG
2585	AGAGGAC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAUA
2588	ACAAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACAAGGC
2591	AGGACAA	CUGAUGAGGCCGAAAGGCCGAA	AGGACAA
2593	ACAGGAC	CUGAUGAGGCCGAAAGGCCGAA	AGAGGAC
2596	CAAACAG	CUGAUGAGGCCGAAAGGCCGAA	ACAAGAG
2601	AAAUUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGAC
2602	GAAAUUG	CUGAUGAGGCCGAAAGGCCGAA	AACAGGA
2607	CCAGUGA	CUGAUGAGGCCGAAAGGCCGAA	AUGCAA
2608	CCCAGUG	CUGAUGAGGCCGAAAGGCCGAA	AAUGCAA
2609	UCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGA
2620	AUAGUGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCC
2626	GCUGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGUGCAA
2628	GAGCUGC	CUGAUGAGGCCGAAAGGCCGAA	AUAGUGC
2635	GAAACUG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCA
2640	UGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUGGAG
2641	CUGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACUGGA
2642	ACUGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACUGG
2653	GGACCCU	CUGAUGAGGCCGAAAGGCCGAA	AUCACUG
2659	CUUGCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCCUGA
2689	CCUCCAA	CUGAUGAGGCCGAAAGGCCGAA	ACCUUGG
2691	GUCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AUACCUU
2700	UGGGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUC
2704	AAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGAGU
2711	CCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
2712	CCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGG
2721	CGCGGAU	CUGAUGAGGCCGAAAGGCCGAA	ACCCUUC
2724	ACAAGCG	CUGAUGAGGCCGAAAGGCCGAA	AUGACCC
2744	CUACACA	CUGAUGAGGCCGAAAGGCCGAA	ACACACA
2750	GCUUGUC	CUGAUGAGGCCGAAAGGCCGAA	ACACAU
2759	AGAGCGA	CUGAUGAGGCCGAAAGGCCGAA	AGCUUGU
2761	ACAGAGC	CUGAUGAGGCCGAAAGGCCGAA	AGAGCUU
2765	GGUGACA	CUGAUGAGGCCGAAAGGCCGAA	AGCGAGA
2769	CCUGGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGAGC
2797	GAAACAU	CUGAUGAGGCCGAAAGGCCGAA	AUUGCAC
2803	UGCAGUG	CUGAUGAGGCCGAAAGGCCGAA	ACCAUGA
2804	CUGCAGU	CUGAUGAGGCCGAAAGGCCGAA	AACCAUG
2813	AGGUCAA	CUGAUGAGGCCGAAAGGCCGAA	ACUGCAG
2815	AAAGGUC	CUGAUGAGGCCGAAAGGCCGAA	AGACUGC
2821	AGCCCAA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAA
2822	GAGCCCA	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCA
2823	UGAGCCC	CUGAUGAGGCCGAAAGGCCGAA	AAAGGUC

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NUC 37793

2829 ADCACUU CUGAUGAGGCCGAAAGGCCGAA AGCCCAA
2837 GUGGGAG CUGAUGAGGCCGAAAGGCCGAA ADCACUU
2840 GAGGUGG CUGAUGAGGCCGAAAGGCCGAA AGGAUCA
2847 GGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUGGG
2853 UACUCAG CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
2860 UCCCAGC CUGAUGAGGCCGAAAGGCCGAA ACTUCAGG
2872 GUGAGCC CUGAUGAGGCCGAAAGGCCGAA AUGGUCC
2877 GUGUUGU CUGAUGAGGCCGAAAGGCCGAA AGCCUAI
2899 AAAADCA CUGAUGAGGCCGAAAGGCCGAA AUUUGCC
2900 AAAAAUC CUGAUGAGGCCGAAAGGCCGAA AAUUGGC
2904 AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AUCAAU
2905 AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAUCAAA
2906 AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAADCAA
2907 AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAADCA
2908 AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAADU
2909 AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAAU
2910 AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAAA
2911 AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAAA
2912 GAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAAA
2913 UGAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAAA
2914 CUGAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAAA
2915 UCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAAA
2916 CUCUGAA CUGAUGAGGCCGAAAGGCCGAA AAAAAAA
2917 UCUCUGA CUGAUGAGGCCGAAAGGCCGAA AAAAAAA
2918 GUCUCUG CUGAUGAGGCCGAAAGGCCGAA AAAAAAA
2919 CGUCUCU CUGAUGAGGCCGAAAGGCCGAA AAAAAAA
2931 GUUGCGA CUGAUGAGGCCGAAAGGCCGAA ACCCGGU
2933 AUGUUGC CUGAUGAGGCCGAAAGGCCGAA AGACCCC
2941 UCUGGGC CUGAUGAGGCCGAAAGGCCGAA AUGUUGC
2951 ACAAGG CUGAUGAGGCCGAAAGGCCGAA AGUCUGG
2952 CACAAAG CUGAUGAGGCCGAAAGGCCGAA AAGUCUG
2955 UAACACA CUGAUGAGGCCGAAAGGCCGAA AGGAAGU
2956 CUAACAC CUGAUGAGGCCGAAAGGCCGAA AAGGAAG
2961 AUUAACU CUGAUGAGGCCGAAAGGCCGAA ACACAAA
2962 UAUAAC CUGAUGAGGCCGAAAGGCCGAA AACACAA
2965 CUUAUU CUGAUGAGGCCGAAAGGCCGAA ACTUACA
2966 GCUUUAU CUGAUGAGGCCGAAAGGCCGAA AACTUAC
2969 AAAGCUU CUGAUGAGGCCGAAAGGCCGAA AUUAACU
2975 GUUGAGA CUGAUGAGGCCGAAAGGCCGAA AGCUUUA
2976 AGUUGAG CUGAUGAGGCCGAAAGGCCGAA AAGCUUU
2977 CAGUUGA CUGAUGAGGCCGAAAGGCCGAA AAAGCUU
2979 GGCAGUU CUGAUGAGGCCGAAAGGCCGAA AGAAAGC

Table 5

Mouse ICAM HH Ribozyme Sequence
nt. Position Ribozyme Sequence

11	CAACGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
23	AGCAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACCACUG
26	AGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAACCA
31	UGUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
34	CGACCCU	CUGAUGAGGCCGAAAGGCCGAA	AUGAGAA
40	AGGCUAC	CUGAUGAGGCCGAAAGGCCGAA	AGUGUGC
48	CCAGGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
54	CCAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCA
58	GGAGCUA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAUG
64	CUGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUG
96	GGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
102	CCAGCAG	CUGAUGAGGCCGAAAGGCCGAA	ACUGGCA
108	GGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
115	AGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAACCA
119	UCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCC
120	GGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
146	GGAAGCG	CUGAUGAGGCCGAAAGGCCGAA	ACGACUG
152	AGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACACAGA
158	GGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACAGGA
165	GCAAAAC	CUGAUGAGGCCGAAAGGCCGAA	ACUUCUG
168	GGGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUU
185	CUGCAGC	CUGAUGAGGCCGAAAGGCCGAA	ACCCACC
209	GCCAGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGGC
227	GCAAAAC	CUGAUGAGGCCGAAAGGCCGAA	ACUUCUG
230	GGAGCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAACUU
237	AGUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AAGCACA
248	UUUAGGA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGUU
253	UCUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGG
263	CAGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAACCCU
267	UAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCCCU
293	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
319	GGCUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCU
335	GUUCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCACAG
337	CAGUGUG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGAC
338	UCAGCUC	CUGAUGAGGCCGAAAGGCCGAA	AACAGCU
359	AGCGGAC	CUGAUGAGGCCGAAAGGCCGAA	ACUGCAC
367	CGGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUU
374	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
375	GGGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUU
378	ACAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAG
386	AAACGAA	CUGAUGAGGCCGAAAGGCCGAA	ACACGGU
394	AGAUCGA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCGG
420	CGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUG
425	CUGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUG

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427 CACUGCU CUGAUGAGGCCGAAAGGCCGAA AGAGCUG
450 GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
451 CAAAGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUUC
456 AGUGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUUAA
495 ACACGGU CUGAUGAGGCCGAAAGGCCGAA AUGGUAG
510 CCCCACG CUGAUGAGGCCGAAAGGCCGAA AGCAGCA
564 GGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACCUGAG
592 CCCAUGU CUGAUGAGGCCGAAAGGCCGAA AUUUUUC
607 CAUGAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGCU
608 GCAUGAG CUGAUGAGGCCGAAAGGCCGAA AAUUGGC
609 GGCAUGA CUGAUGAGGCCGAAAGGCCGAA AAUUGG
611 GCGGCAU CUGAUGAGGCCGAAAGGCCGAA AGAAAUU
656 CAGCUCA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
657 UCAGCUC CUGAUGAGGCCGAAAGGCCGAA AACAGCU
668 GGUGGCC CUGAUGAGGCCGAAAGGCCGAA AGGCUUG
677 AGGCUUG CUGAUGAGGCCGAAAGGCCGAA AGAGGUC
684 AGGACCG CUGAUGAGGCCGAAAGGCCGAA AGCUGAA
692 AAGAUUG CUGAUGAGGCCGAAAGGCCGAA AAGUCCG
693 GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
696 GAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGG
709 UGAGGUG CUGAUGAGGCCGAAAGGCCGAA AGCCGCC
720 AGCUGAA CUGAUGAGGCCGAAAGGCCGAA AGUUGUA
723 CGGAGCU CUGAUGAGGCCGAAAGGCCGAA AAAAGUU
735 UCUCCAG CUGAUGAGGCCGAAAGGCCGAA AUUUGGU
738 CCAUCAC CUGAUGAGGCCGAAAGGCCGAA AGGCCCA
765 GGAAGCG CUGAUGAGGCCGAAAGGCCGAA ACAGCUG
769 GGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
770 UUCGAGG CUGAUGAGGCCGAAAGGCCGAA AGCAAAA
785 GGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
786 AGGCAGG CUGAUGAGGCCGAAAGGCCGAA AACAGGC
792 CUUCCGA CUGAUGAGGCCGAAAGGCCGAA ACCUCCA
794 AGUCUCC CUGAUGAGGCCGAAAGGCCGAA AGCCAG
807 CCAGGUA CUGAUGAGGCCGAAAGGCCGAA AUCCGAG
833 GGGUGUC CUGAUGAGGCCGAAAGGCCGAA AGCUUUG
846 CAACGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
851 GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AGGUUUC
863 CCAGAGG CUGAUGAGGCCGAAAGGCCGAA AGUGGCU
866 GGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCUUC
867 UCUCGG CUGAUGAGGCCGAAAGGCCGAA AACGAAU
869 CUUGCAU CUGAUGAGGCCGAAAGGCCGAA AGGAAGA
881 ACGGUUU CUGAUGAGGCCGAAAGGCCGAA AAGCCAU
885 UCACCU CUGAUGAGGCCGAAAGGCCGAA ACCAAGG
933 CCAGAAU CUGAUGAGGCCGAAAGGCCGAA AUUAUAG
936 GCACCAG CUGAUGAGGCCGAAAGGCCGAA AUGAUUA
978 AGUUGUA CUGAUGAGGCCGAAAGGCCGAA ACUGUUA
980 AAAGUUG CUGAUGAGGCCGAAAGGCCGAA AGACUGU
986 AGCUGAA CUGAUGAGGCCGAAAGGCCGAA AGUUGUA
987 GAGCUCA CUGAUGAGGCCGAAAGGCCGAA AAGUUGU
988 GGAGCUG CUGAUGAGGCCGAAAGGCCGAA AAGUUG

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1005	UCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGU
1006	UUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCA
1023	CUUCCGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
1025	CCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACCTUC
1066	UUADUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAGUGG
1092	GGCCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCAGU
1093	UUGGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCA
1125	UCAAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGG
1163	GCAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCG
1164	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUC
1166	AGAGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCU
1172	GGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACAGGA
1200	UGUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
1201	CUGUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGC
1203	ACUGGUG	CUGAUGAGGCCGAAAGGCCGAA	AAAAGU
1227	GCACACG	CUGAUGAGGCCGAAAGGCCGAA	AUGUACC
1228	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUC
1233	CUUCUCG	CUGAUGAGGCCGAAAGGCCGAA	AAACGAA
1238	AGGACCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAC
1264	CUUGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCCUUC
1267	UUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCA
1294	GGCUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCU
1295	CUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCCUUC
1306	CADUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUGC
1321	UCCUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUUC
1334	UUUAGGA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGUU
1344	CACUCUC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCAU
1351	UAACUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCA
1353	CACCUUC	CUGAUGAGGCCGAAAGGCCGAA	ACCCACU
1366	AGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	ACUGUUA
1367	AGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGCU
1368	AGAGUGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUAC
1380	CCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
1388	AGCCACU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCC
1398	GUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGCCA
1402	AGUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AAGCACA
1408	CCUCCCC	CUGAUGAGGCCGAAAGGCCGAA	AUCUCGC
1410	CCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACCTUC
1421	ACAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGG
1425	CUCUACC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGU
1429	CAGGGGC	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGA
1444	UCCUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUUC
1455	UCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCC
1482	GGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AACAACU
1484	CAUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAACAG
1493	GUUCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCACAG
1500	GGACCAU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCAU
1503	GAAUGAU	CUGAUGAGGCCGAAAGGCCGAA	AUAGUCC
1506	CGGUUAU	CUGAUGAGGCCGAAAGGCCGAA	AACAUAU

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1509	ACACGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAG
1518	CGCCUGG	CUGAUGAGGCCGAAAGGCCGAA	ACCAUGA
1530	CCAGAAU	CUGAUGAGGCCGAAAGGCCGAA	AUUAUAG
1533	GGCCAC	CUGAUGAGGCCGAAAGGCCGAA	AUGACCA
1551	AGCUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCAUG
1559	AGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGCU
1563	GGUUAUA	CUGAUGAGGCCGAAAGGCCGAA	ACUAUAG
1565	GCGGUUA	CUGAUGAGGCCGAAAGGCCGAA	AAACAUU
1567	UGGCGGU	CUGAUGAGGCCGAAAGGCCGAA	AUAACA
1584	AUAUCCU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUC
1592	UAACUUG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCCU
1599	CCUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AACUUGU
1651	GCUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGG
1661	CAAAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUUC
1663	UUCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGUU
1678	CCAGGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
1680	CCAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGCU
1681	GCCAGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGGC
1684	ACAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGU
1690	AGAUCGA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCGG
1691	AAGAUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCG
1696	CCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
1698	CUCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCCG
1737	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUC
1750	UGAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCGCC
1756	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
1787	UGGGGAC	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUC
1790	AUUAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACAAUGC
1793	UCCAGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGACCA
1797	UUUAUGU	CUGAUGAGGCCGAAAGGCCGAA	ACUGGUG
1802	UCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGU
1812	GGCCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCAGU
1813	UGAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AAUGCUG
1825	GCAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGG
1837	GGAGCUA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAUG
1845	GGUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGCUCC
1856	AAGAUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCG
1861	UACUGGA	CUGAUGAGGCCGAAAGGCCGAA	AUCAUGU
1865	CUGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGUG
1868	UUUAUGU	CUGAUGAGGCCGAAAGGCCGAA	ACUGGUG
1877	AGCUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCAUG
1901	GUCCCUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUA
1912	ACUGAUC	CUGAUGAGGCCGAAAGGCCGAA	ACUAUAU
1922	UAACUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCA
1923	GAUAACU	CUGAUGAGGCCGAAAGGCCGAA	AGCAUCA
1928	CUGGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUAA
1930	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAACUCU
1964	UGGGGAC	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUC
1983	UAACUUG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCCU

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1996	GGCUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCU
2005	GGUCCGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCA
2013	UACUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGC
2015	CCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
2020	CUCAGAA	CUGAUGAGGCCGAAAGGCCGAA	AACCAAC
2039	CCUCUGC	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGC
2040	CCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
2057	GGAUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCA
2061	ACACGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAG
2071	CUGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGUG
2076	UAGCUCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCUAC
2097	CAUCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
2098	CGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUG
2115	AUCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGC
2128	CUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCUG
2130	GAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
2145	CAUCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
2152	AACUCUA	CUGAUGAGGCCGAAAGGCCGAA	AUUAUA
2156	UAUAUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAA
2158	AUUAUA	CUGAUGAGGCCGAAAGGCCGAA	AUACAUC
2159	AAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2160	AAAUUA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2162	CUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAUA
2163	AAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2166	AAUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAGU
2167	AAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2170	CUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAUA
2171	GGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AACAACU
2173	CUGGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUAA
2174	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUA
2175	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAACUCU
2176	UAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAAACUC
2183	CAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGU
2185	CUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCUG
2186	ACUCAU	CUGAUGAGGCCGAAAGGCCGAA	AADAGCU
2187	UACUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGC
2189	GGUACUC	CUGAUGAGGCCGAAAGGCCGAA	AUAAUA
2196	CAUCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
2198	AACADAA	CUGAUGAGGCCGAAAGGCCGAA	AGGCUGC
2199	AUAAACA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGGC
2200	CUUGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGA
2201	GCCGACA	CUGAUGAGGCCGAAAGGCCGAA	AAAACUU
2205	UCAGGCC	CUGAUGAGGCCGAAAGGCCGAA	ACAUAUA
2210	AGCCACU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCC
2220	AGAGAAC	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
2224	GAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUAG
2226	GCGGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCA
2233	CCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
2242	GGUCCGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCA

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2248	UGGGAUG	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUA
2254	UCAGUGU	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGA
2259	CACCGUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUGAU
2260	GCACCGU	CUGAUGAGGCCGAAAGGCCGAA	AAUGUGA
2266	UCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCC
2274	UCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGU
2279	CUUGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCUUUC
2282	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
2288	AGGCCAU	CUGAUGAGGCCGAAAGGCCGAA	ACUUAUA
2291	AGCAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACCACUG
2321	CCCADGU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUC
2338	CAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCA
2339	CAAAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUUC
2341	AGGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUC
2344	GCUGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAAA
2358	CUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
2359	UCUGUUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGCAG
2360	UUCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGUU
2376	UCAGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACCACCU
2377	CUCAGAA	CUGAUGAGGCCGAAAGGCCGAA	AACCACC
2378	CAGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAACCCU
2379	CUUAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGCA
2380	GCCGACA	CUGAUGAGGCCGAAAGGCCGAA	AAAACUU
2382	GGGGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAU
2384	UUGUGUC	CUGAUGAGGCCGAAAGGCCGAA	ACUGGAU
2399	GUCCACA	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUU
2401	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
2411	GCAUCCU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGUA
2417	ACGU AUG	CUGAUGAGGCCGAAAGGCCGAA	ACCAUUC
2418	GGCCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCAGU
2425	AACCCUC	CUGAUGAGGCCGAAAGGCCGAA	ACCCAUG
2426	AAACUCU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUU
2433	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUA
2434	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAACUCU
2448	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
2449	GGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUU
2451	AGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGGC
2452	GAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
2455	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
2459	GGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGUGG
2460	CGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUG
2479	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUC
2480	GCAUCAC	CUGAUGAGGCCGAAAGGCCGAA	ACGGUGA
2483	GGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGG
2484	GACUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAAAAAG
2492	AGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGCU
2504	ACAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGG
2508	UGGGAUG	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUA
2509	CUGGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUAA

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2510 GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AACUCUA
2520 CAUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAAAAG
2521 UGAGGGU CUGAUGAGGCCGAAAGGCCGAA AADGCTG
2533 GAUACCU CUGAUGAGGCCGAAAGGCCGAA AGCAUCA
2540 CACAGCG CUGAUGAGGCCGAAAGGCCGAA ACUGCUG
2545 AGGACCA CUGAUGAGGCCGAAAGGCCGAA ACAGCAC
2568 UUUGACA CUGAUGAGGCCGAAAGGCCGAA ACUUCAC
2579 CAGGCCA CUGAUGAGGCCGAAAGGCCGAA AACUUAU
2585 AGAGAAC CUGAUGAGGCCGAAAGGCCGAA ADGCCAG
2588 AUUAGAG CUGAUGAGGCCGAAAGGCCGAA ACAAUUC
2591 AGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGAACCA
2593 GCAGAGC CUGAUGAGGCCGAAAGGCCGAA AAAGAAG
2596 CADUGGG CUGAUGAGGCCGAAAGGCCGAA ACAAAAG
2601 AAACGAA CUGAUGAGGCCGAAAGGCCGAA ACACGGU
2602 GGGAUUG CUGAUGAGGCCGAAAGGCCGAA AGCUGGA
2607 CCAGGUA CUGAUGAGGCCGAAAGGCCGAA ADCCGAG
2608 CACAGCG CUGAUGAGGCCGAAAGGCCGAA ACUGCUG
2609 UCCUGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUCU
2620 GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
2626 GCUGGAA CUGAUGAGGCCGAAAGGCCGAA AUCGAAA
2628 AGGCUAC CUGAUGAGGCCGAAAGGCCGAA AGUGUGC
2635 AGGACCG CUGAUGAGGCCGAAAGGCCGAA AGCUGAA
2640 GGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
2641 CUGCUGA CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
2642 GAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGG
2653 GCAUCCU CUGAUGAGGCCGAAAGGCCGAA ACCAGUA
2659 CUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCCUUC
2689 CCUCGGA CUGAUGAGGCCGAAAGGCCGAA ACAUUAG
2691 GGCCUCG CUGAUGAGGCCGAAAGGCCGAA AGACAUU
2700 GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCUUC
2704 AGGCUGG CUGAUGAGGCCGAAAGGCCGAA AGAGGUC
2711 CUGCUGA CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
2712 CCCUUCU CUGAUGAGGCCGAAAGGCCGAA AGACCTC
2721 CUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCCUUC
2724 GCACACG CUGAUGAGGCCGAAAGGCCGAA AUGUACC
2744 CUGCACG CUGAUGAGGCCGAAAGGCCGAA ACCCACC
2750 GGUACUC CUGAUGAGGCCGAAAGGCCGAA ADAAAUU
2759 AGAUCGA CUGAUGAGGCCGAAAGGCCGAA AGUCCCG
2761 GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
2765 AGCGGCA CUGAUGAGGCCGAAAGGCCGAA AGCAAAA
2769 CCUGUUU CUGAUGAGGCCGAAAGGCCGAA ACAGACU
2797 GGACCAU CUGAUGAGGCCGAAAGGCCGAA AUUUCAU
2803 CGCCUGG CUGAUGAGGCCGAAAGGCCGAA ACCAUGA
2804 CUGCACG CUGAUGAGGCCGAAAGGCCGAA ACCCACC
2813 GGGUCAG CUGAUGAGGCCGAAAGGCCGAA ACCGGAG
2815 AAAGUUG CUGAUGAGGCCGAAAGGCCGAA AGACUGU
2821 CCUCCAG CUGAUGAGGCCGAAAGGCCGAA AGGUACG
2822 AAGUCCG CUGAUGAGGCCGAAAGGCCGAA AGGCUCC
2823 UGGGAGC CUGAUGAGGCCGAAAGGCCGAA AAAGGCA

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2829	AUGAUUA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAG
2837	UCAGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACCACCU
2840	CAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCA
2847	GGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGG
2853	AACAUAA	CUGAUGAGGCCGAAAGGCCGAA	AGGCUGC
2860	UCACAGU	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGC
2872	CUUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCC
2877	GUGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCGSAA
2899	AAGAUCG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCG
2900	AAAACUC	CUGAUGAGGCCGAAAGGCCGAA	AAAUUAA
2904	AAUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAGU
2905	CAAUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAG
2906	UAUUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAA
2907	AAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2908	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUC
2909	AGAGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCU
2910	AAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2911	AAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2912	GACAUUA	CUGAUGAGGCCGAAAGGCCGAA	AGAACAA
2913	UGACCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAA
2914	CUUAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGCA
2915	UCUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
2916	CUCCGGA	CUGAUGAGGCCGAAAGGCCGAA	ACGAUUA
2917	UCUCCGG	CUGAUGAGGCCGAAAGGCCGAA	AACGAUU
2918	CUCUCCG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCAA
2919	CGACCCU	CUGAUGAGGCCGAAAGGCCGAA	AUGAGAA
2931	CUUCCGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
2933	CCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACCUU
2941	UGGGGAC	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUC
2951	GCAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGCGUGG
2952	CACAGCG	CUGAUGAGGCCGAAAGGCCGAA	ACUGCUG
2955	UGACACA	CUGAUGAGGCCGAAAGGCCGAA	AGUCACU
2956	UUGAUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGGAAA
2961	AGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACACAGA
2962	AAUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2965	CUUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCAAA
2966	CCUCUGC	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGC
2969	AAAACUU	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUU
2975	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUA
2976	AGUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AACCCUC
2977	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCTU
2979	GGCAUUA	CUGAUGAGGCCGAAAGGCCGAA	AGAAUGA

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Table 6
Human ICAM Hairpin Ribozyme/Substrate Sequences
nt. Position
Hairpin Ribozyme Sequence

Substrate

70	GGGCGGG AGAA GCTG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CAGCA GCC CCGGGCC
86	GGAGUGCG AGAA GCGC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	GCUCU GCC CGCACUCC
343	CCCAUCAG AGAA GUUU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	AAACU GCC CUGAUGGG
635	GCCCTUGG AGAA GCAG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CUGCG GCC CCAAGGGC
653	UGUUCUCA AGAA GCTC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	GAGCU GUU UGAGAACA
782	AGACUGGG AGAA GCGC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	GGGCU GUU CCGAGUCU
920	CUGCACAC AGAA GCGG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CGGCU GAC GUGUGCAG
1301	ACAUUGGA AGAA GCTG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CAGCA GAC UCCAAUGU
1373	CCCCGAUG AGAA GUGG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CCACU GCC CAUGGGG
1521	AUGACTGC AGAA GCUA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UAGCA GCC GCAGUCAU
1594	CUGTUGUA AGAA GUAU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	AUACA GAC UACACACG
2008	ACCCAAUA AGAA GCAA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UUUCU GCC UAUUGGUG
2034	UTCUGUAA AGAA GUGG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CCACA GAC UUACAGAA
2125	GGUCAGUA AGAA GCAG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CUGCU GGC UACUGACC
2132	GGGUUGGG AGAA GUAG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CUACU GAC CCCAACCC
2276	ACCUGUAC AGAA GUAC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	GUACA GUU GUACAGGU
2810	AAGGUCAA AGAA GCAG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CUCCA GUC UUGACCUU

Table 7
Mouse ICAM Halpin Ribozyme/Substrate Sequences

Halpin Ribozyme Sequence

Substrate

nt.
Position

76	GGGAUCAC AGAA GUUA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UCACC GUU GUGAUCCC
164	UGAGGAG AGAA GUUC ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	GAACU GUU CUUCCUCA
252	UCAGCUCA AGAA GCUU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	AAGCU GUU UGAGCTUGA
284	GCACAGCG AGAA GCUU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CAGCA GUC CGCUGUGC
318	AAGCGGAC AGAA GCAC ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	GUGCA GUC GUCGCGCU
447	AGAGCTUG AGAA GCGG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CCGCG GAC CCAGCUCU
804	UCUCCUG AGAA GCAU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	AUGCC GAC CCAGGAGA
847	UTUACCA AGAA GUGG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CCACU GCC UUGGUAGA
913	AGGAUCUG AGAA GCUA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UAGCG GAC CAGAUCCU
946	AAGUUGUA AGAA GUUA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UAAAC GUC UACAACUU
1234	CCCAAGCA AGAA GUCU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	AGACG GAC UGCTUUGG
1275	AUUUCAGA AGAA GCUU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CAGCA GAC UCTGAAAU
1325	UGCCUUC AGAA GCAG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CUCCA GAC GGAAGGCA
1350	CCCGAUG AGAA GCAG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CUCCU GCC CAUCGGGG
1534	ACAUAGA AGAA GCCA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UGGCA GCC UCUUAUGU
1851	GUCCACCG AGAA GUAG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CUACA GCC CGGUGGAC
1880	AGAAUGAA AGAA GCGU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	ACGCU GAC UUCAUUCU

Table 8
Rat ICAM Hairpin Ribozyme/Substrate Sequences

Position nt.	Hairpin Ribozyme Sequence	Substrate
5	AAAGUGCA AGAA GCAG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CUGCU GCC UGCACUUI
59	GGAGCAGA AGAA GCAU ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	AUGCU GCC UUGCUCC
84	GGGAUCAC AGAA GCGA ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	UCGCC GUU GUGAUCCC
295	GCACAGUG AGAA GCTG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CAGCA GAC CACUGUGC
329	AAGCCGAG AGAA GCGU ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	ACGCA GUC CUCGGCTU
433	UCCACCA AGAA GCGC ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	GGCTU GGC UGGUGGAA
626	CAUUCUUG AGAA GUGA ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	UCACU GUU CAAGAUG
806	UUCCCAGG AGAA GCAU ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	AUGCU GAC CCUGGAGA
849	UCCACUGA AGAA GUGG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CCACU GCC UCAGUGGA
915	AGGUCUG AGAA GCCA ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	UGGCG GAC CAGACCTU
1182	ACCUCCAA AGAA GCAG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CUGCG GGC UUGGAGGU
1307	AUGUAAGA AGAA GCTG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CAGCA GAC UCTUACAU
1357	UGCUTUCC AGAA GCAG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CUCCA GCC GGAAGCA
1382	UCCCGAUA AGAA GCGG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CCGCU GCC UAUCCGGA
1858	GCCACCA AGAA GUAG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CUACA GCC UGGUGGCG
1887	AGNAGAA AGAA GCTU ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	AGGCU GAC UUCUUCU
2012	GAGUUGGG AGAA GUGU ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	ACACU GUC CCCAACUC
2303	AGACUCCA AGAA GUGG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CCACA GCC UGGAGUCU
2539	CCUCCAC AGAA GCUU ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	AAGCU GUU GUGGAGG

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Table 9: Rat ICAM HH Ribozyme Target Sequence

nt. Position	HH Target sequence	nt. Position	HH Target Sequence
11	GAUCCAAU U CACACUGA	394	GUGGUGCU U CUGAACAG
23	GCUGACUU C CUUCUCUA	420	GCACCCCU C CCAGCGCA
26	GAACUGCU C UUCUCUU	425	CCUGGCU U CCGCCACC
31	CCUCUGCU C CUGGUCU	427	UCCUGUU U AAAAACCA
34	CUGAAGCU C AGAUUAC	450	AAGAACCU C AUCCUGCG
40	CUCAAGU A CAGCCCC	451	GGGUACUU C CCGCAGGC
48	GAGAACCU C GGCUCGG	456	CUUGGCU C UGACCCA
54	CCCGCCU C CUGAGCC	495	GCCACCAU C ACUGUGUA
58	CGUGCCU U UAGCUC	510	GUGCUGCU C CGUGGGA
64	CAUUGCU U CAACCCU	564	GAAAUGU U CCAACCAC
96	CCUCUGCU C CUGGUCU	592	GGGAGUAU C ACCAGGGA
102	CUCCUGGU C CUGGUCG	607	GAGCCAAU U UCUCAGC
108	GGACUGCU U GGGGAACU	608	AGCCAAU U UCUCAGC
115	UCCUACCU U UGUUCCA	609	GCCAAUU C UCAGGCU
119	GACACUGU C CCAACUC	611	CAAUUCU C AUGCUCA
120	GUUGUGAU C CCGGGCC	656	GUCACUGU U CAGAAUG
146	CCAGACCU U GGAACUC	657	UCACUGU C AAGAAUG
152	ACCGGCU C CACCUCA	668	GAACUGCU C UUCUCU
158	AUUUCUU C ACGAGUA	677	GCACCCU C CCAGCGCA
165	UGAACAGU A CUUCCCC	684	AGGCAGCU C CGGACUU
168	GAAGCCU C CUGGUCG	692	CCAGACCU U GGAACUC
185	GGGUGAU C CGGCGAG	693	CGGACUU C GACUUC
209	CAGCCCU A AUUCAGC	696	GCCUGUU C CUGGUCU
227	GACCAAGU A ACUGUGA	709	CAGCAUU A CCCCUC
230	CAAGCUGU U GUGGGAG	720	CUACAACU U UCAGCUC
237	CUGAAGCU C GACACCC	723	CAACUUU C AGCUCCA
248	GGCCCCU A CCUAGGA	735	CUCCUGGU C CUGGUCG
253	CACUGCCU C AGUGGAG	738	UCCUGCCU C GGGUGGA
263	GAGCCAAU U UCUCAGC	765	ACUGUGCU U UGAGAACU
267	GAAGCCU C CUGGUCG	769	UCUUGUGU U CCGGGA
293	GAAGCUCU U CAGCUGA	770	CUUGUGU C CCUGGAG
319	CGGAGAU C ACAAACA	785	AGGCUGU U UCCUGCCU
335	ACUGUGCU U UGAGAACU	786	GGCCUGU U CUGGUCU
337	UGUGCUA A UGUCCUC	792	CUCCUGGU C CUGGUCG
338	AAGCUCU C AAGCUGAG	794	UCCUGCCU C UGAAGCUC
359	CACGCAGU C CUGGCU	807	GCUCAGAU A UACUGGA
367	CAUUGCU U CAACCCU	833	CCUGGGU U GAGACUA
374	UUACCCU C ACCACCU	846	CUGACAGU U AUUUAUG
375	AGAAGCCU U CCUGCUC	851	GCUCACCU U UAGCAGCU
378	ACCCACCU C ACAGGUA	863	CAUUGCU U CAACCCU
386	CGCUGUGU U UUGAGCU	866	CCAUGCU C CUCGACA

867	GACCACCU C CCCACCTA	1421	GGGUACCU C CCCCAGGC
869	CUCUCCU C UUGGGAAG	1425	ACCCACCU C CUCUGGCU
881	AADGGCUU C AACCGUG	1429	ADACUUGU A GCCUCAGG
885	GACCAAGU A ACUGUGAA	1444	AGAAGGCU C AGGAGGAG
933	UGUGUADU C GUUCCAG	1455	GGGAGUAD C ACCAGGGA
936	GCAGAGAU U UUGUGUCA	1482	AGGGUACU U CCCCAGG
978	UUGAGAAU C UACAACUU	1484	ACUGCUU C CCUCUUGC
980	GAGAAUCU A CAACUUU	1493	CCUGGGU U GGAGACUA
986	CUACAACU U UUCAGCUC	1500	CGUGAAAU U AUGGUCAA
987	UACAACUU U UCAGCUC	1503	GAAAUUGU U CCACCCAC
988	ACAACUUU U CAGCUC	1506	UGGUCAU A AUUGUUGG
1005	UUCGUGAU C GUGGUGUC	1509	GCCACCAU C ACUGUGUA
1006	GUGGGAGU A UCAACAGG	1518	GUCCUGGU C GCCGUGU
1023	CCGGAGGU C UCAGAGG	1530	ACCUGGGU C ADAAUUGU
1025	GGAGGUCU C AGAAGGGG	1533	CUGAUCAD U GCGGGCUU
1066	CCUACCUU U GUUCCAA	1551	GUGGCUU C UGCUCGUA
1092	AGAGGGGU C UCAGCAGA	1559	UGGGAAGU C CCUGUUUA
1093	AGGGGAU C CAGCCCU	1563	UCCUACU U UGUUCCCA
1125	CCCCACU C UUGUUGAU	1565	UUACACU A UUACCGCC
1163	ACGAGCU U CUUUGCU	1567	ACACCUAU U ACOCAG
1164	CGACGCUU C UUUUGCUC	1584	AGGAAGAU C AGGAUAUA
1166	ACGCUUCU U UUGCUC	1592	CAGGAUAD A CAAGUAC
1172	CUUUGCU C UGCGCCU	1599	UACAAGUU A CAGAAGGC
1200	AUCCAADU C ACACGAA	1651	CCCGCCU C CCUGAGCC
1201	UUGGGCUU C UCCACAGG	1661	CUGCAU U GCCCUGGU
1203	GGGCUUCU C CACAGGUC	1663	GAACAGAU C AAUGGACA
1227	UUGGAACU C CAUGGCU	1678	GAGAACCU C GGCCUGGG
1228	GCGGGCUU C GUGAUCU	1680	GGGCUUCU C CACAGGUC
1233	CUCCUGGU C CUGGUC	1681	GGCCUGU U CCUGCCUC
1238	UGUGCUAU A UGGUCCUC	1684	CUGCUGU A GACCCUC
1264	GAAAGAU C ADACGGU	1690	CCCCACU A CAUACAU
1267	GUACUGU U CAAGAUG	1691	CCGACU U CGAUCUUC
1294	CAGAGAUU U UGUGCAG	1696	CUCCUGGU C CUGGUC
1295	AGAGGGGU C UCAGCAGA	1698	UCAGAUAD A CCUGGAGA
1306	AGCAGACU C UACADGC	1737	GAUCACAU U CACGGUC
1321	AACAGAGU C UGGGAAA	1750	GUCCAUU A CACCUAU
1334	GUADUCU U CCGAGAGC	1756	CCUCUGCU C CUGGUCCU
1344	UCGGUGCU C AGGUADCC	1787	GAGAACCU C GGCCUGGG
1351	UCAGGCU A AGAGGACU	1790	GACACUGU C CCAACUC
1353	UAGCAGCU C AACADGG	1793	AUGGUCCU C ACCUGGAC
1366	AGGGUACU U CCCCAGG	1797	UCCUGUU U AAAAACCA
1367	GGGUACCU C CCCCAGGC	1802	GUUCAGAU A UACCGGA
1368	GAUGGUGU C CCGCUGCC	1812	AACAGAGU C UGGGAAA
1380	CUGCCUAD C GGAUGGU	1813	GCGGGCUU C GUGAUCU
1388	UGGAGACU A ACUGGAUG	1825	GCCACCAU C ACUGUGUA
1398	CUGGCGU C ACAGGACA	1837	ACCCACCU C ACAGGGUA
1402	CUGUGCUU U GAGAACUG	1845	AGAGGACU C GGAGGGGC
1408	UUCGUGAU C GUGGUGUC	1856	CCCCUAAU C UGACCUGC
1410	CGAACUAD C CAGUGGAC	1861	CAUGUGCU A UAUGGUCC

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1865	UAUCCGGU A GACACAAG	2198	GAAUGUCU C CGAGGUCA
1868	UCACGAGU C AUADAADU	2199	AGACUCUU A CAUGCCAG
1877	ACAGUACU U CCCCCAGG	2200	GGGUACUU C CCCCAGGC
1901	CUAAAACU C AAGGUACA	2201	GGGCUUCU C CACAGGUC
1912	GAACAGAU C AAUGGACA	2205	UUUUGUGU C AGCCACTG
1922	AUGUAAGU U AUUGCCUA	2210	UGGAGACTU A ACUGGADG
1923	UGGACGCU C ACCUUUAG	2220	GAGAAACU C GGCCUGGG
1928	GUCACAGU A UACCUUGA	2224	ACAUAACU U CCUACCUU
1930	UGGAGACTU A ACUGGADG	2226	CUGGACCU C AGGCCACA
1964	AGAGAUUU U GUGUCAGC	2233	UCAUGCUU C ACAGAACU
1983	GAGAAACU C GGCCUGGG	2242	ACACAGCU C UCAGUAGU
1996	UGGAAGCU C UUCAAGCU	2248	CUCCUGGU C CUGGUCGC
2005	AUGUAAGU U AUUGCCUA	2254	AUCCAAUU C ACACUGAA
2013	CGCUGCCU A UCGGGAUG	2259	GAUCACAU U CAUGGUGC
2015	CUGCCUAAU C GGGADGGU	2260	AUCACAUU C ACGGUGCU
2020	UAUUGAGU A CUCUGUAC	2266	AUCAGGAU A UACAAGUU
2039	CGGAGGAU C ACAAACGA	2274	GAGCAGGU U AACADGUA
2040	CCUGACCU C CUGGAGGU	2279	GGAAAGAU C AUACGGGU
2057	CUGGUCCU C CAUUGGCU	2282	ACAGUUAU U UAUUGAGU
2061	GCGUCCAU U UACAACUA	2288	GCCCCGGU C CUCCAAUG
2071	AUACUUGU A GGCUCAGG	2291	CAGGADAU A CAAGUUAU
2076	UGUAGCCU C AGGCCUAA	2321	GGAAAGAU C AUACGGGU
2097	CCAACUCU U GUUGAUGU	2338	UUGGGCUU C UCCACAGG
2098	CCUGACCU C CUGGAGGU	2339	GGGUACUU C CCCCAGGC
2115	UUCOGACU A GGGUCCUG	2341	GGGCUUGU C GGUGCUCA
2128	AGUGCUGU A CCAUGAUC	2344	CUGCUCGU A GAUCUCUC
2130	GCCUGUUU C CGCCUCU	2358	CCUGCCU C CUCCACA
2145	CCAACUCU U GUUGAUGU	2359	CCAUCCAU C CCACAGAA
2152	UUGAGAAU C UACAACUU	2360	CUUGUGUU C CCUGGAAG
2156	UGACAGUU A UUAUUGA	2376	GAACUGCU C UUCUCUU
2158	UGAUGUAU U UAUUAUU	2377	GACUUCU U CUCUAUA
2159	GAUGUAUU U AUUAUUUC	2378	GCUGAUUU C UUCACGA
2160	AUGUAUUU A UUAADUCA	2379	CUGCUCUU C CUCUUGCG
2162	ACAUUCU A CCUUUGUU	2380	UGAUUUUU U UCAAGAGU
2163	UAUUUAUU A AUUCAGAG	2382	AUUUCUUU C ACGAGUCA
2166	UGAUGUAU U UAUUAUU	2384	UAUCCGGU A GACACAAG
2167	GAUGUAUU U AUUAUUUC	2399	UAAAUACU A UGUGGACG
2170	GUUUUUUU U AAUUCAGA	2401	UGUGCUAU A UGUUCCUC
2171	CAGUUAUU U AUUGAGUA	2411	CAUUUUUU C AUGCUUCA
2173	UGUGCUAU A UGUUCCUC	2417	AUCAGGAU A UACAAGUU
2174	UCUCUAUU A CCCCUGCU	2418	UCAUGCUU C ACAGAACU
2175	AUUUCUUU C ACGAGUCA	2425	UUUUUAUU U CAGAGUUC
2176	GAAAAGUU U CCAACCAC	2426	CCUGGGGU U GGAGACUA
2183	UGACAGUU A UUAUUGA	2433	UCAGAGUU C UGACAGUU
2185	ACAGUUAU U UAUUGAGU	2434	CGGAGGAU C ACAAACGA
2186	CAGUUAUU U AUUGAGUA	2448	UGAACAGU A CUUCCCCC
2187	AGUUAUUU A UUGAGUAC	2449	GAAGCCUU C CUGCCUCG
2189	UUUUUUUU U GAGUACCC	2451	GGCCUGUU U CCUGCCUC
2196	CUGACAGU U AUUUUUUG	2452	GCCUGUUU C CUGCCUCU

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2455	ACAUUCU A CCUUGUU	2761	CGGACUU C GAUCUUC
2459	CCUGGCU C CUCCACA	2765	CUUUGCU C UGCGGCU
2460	CCUACCU U GUUCCAA	2769	UUCUCU U ACCCCUG
2479	UACACCU A UUACCGC	2797	CGUGAAU U AUGGUC
2480	GUUGCGU U GUGAUCC	2803	CUCAUGU U CACAGAC
2483	ACCUUGU U CCACUGU	2804	UCAUGCU C ACAGACU
2484	CCUUGUU C CCACUGU	2813	GCUCUCC C CGGACCU
2492	GACCACT C CCACCUA	2815	CGGACUU C GAUCUUC
2504	ACCUACU A CAUCCUA	2821	CCUGACU C CUGGAGU
2508	ACAACAU U CCUACCU	2822	UACAACU U UCAGUCC
2509	CAUACAU C CUACCUU	2823	CAACUUU C AGCUCCA
2510	GUCAUUU A CACCUAU	2829	UUGGUGU C AGGUUCC
2520	ACCUUGU U CCACUGU	2837	CACAGGU A CUUCCCC
2521	CCUUGUU C CCACUGU	2840	GCACCCU C CCAGCGC
2533	ACAGCAU U ACCCUCA	2847	UUAACCU C ACCACCU
2540	UUGGUGU C AGGUUCC	2853	UUCGACU U CCGACU
2545	AGGAGCU C CGGACUU	2860	UCUUGU C CCUGGAA
2568	CAGACAU U UGUGCAG	2872	GGGCGU C GGUCUCA
2579	CCUGACU U UGCGUGG	2877	UGGAGU C CCAGCAC
2585	CUGCGU A GACUUC	2899	AGGAGCU C CGGACUU
2588	UGCUCCU C CCACAGC	2900	GGCUGU U CCUCUCU
2591	CUUUCU C UUGGAG	2904	GAACUGU C UUCUCU
2593	UCUUAU A CCGUGCU	2905	GGCUGU U CCUCUCU
2596	CUUGGU C CUGGUGC	2906	GUUGAU A UUAUAUA
2601	UGGCUAU A UGUCUCC	2907	CUGUCU C CUUUGCG
2602	GUUGGU C GCGUGU	2908	UGAUGAU U UAUUAU
2607	GUGGAGU A UCACAGG	2909	GAACUGU C UUCUCU
2608	CUUAGCU C CCGUGGA	2910	ACUUCU C UCUAUAC
2609	UGGAGAU A ACUGGAG	2911	UUCUUCU C UAUUACC
2620	UCAGAU C UGACAGU	2912	AUGUAUU A UUAUAUA
2626	CUUCAGU A GUGUGCU	2913	UGUGAU C GUUCCAG
2628	UACAACU U UCAUCUC	2914	GUADUAU U AAUUCAG
2635	UCACAGU C CAUUCAC	2915	UAUUAU A AUUCAGG
2640	GUUCAGU A UCCAUCC	2916	CUUUCU C UUGGAG
2641	CCCAACU A CAUACAU	2917	CUUUCU U GCGAGAC
2642	GCCUGUU C CUGUCU	2918	AUUUCUU C ACGAGUA
2653	CCACAGU C AGGUGCU	2919	UUUUGU C AGCCACU
2659	AGAAGGU C CUGCAAG	2931	GAUGGUG C CCGUGGC
2689	ACUAGGU C CUGAGCU	2933	UGGAGU C CCAGCAC
2691	UCAGGCU A AGAGGAC	2941	CAGUACU C CCGAGGC
2700	AGGUACU U CCGCAGG	2951	ACCAUGU U CCUCUG
2704	GACCACT C CCACCUA	2952	CCGACU U CGAUCUC
2711	CCUACCU U AGGAGGU	2955	UGCUUCU C UGACUGG
2712	CCUACCU A GGAAGGU	2956	CUUUCU U GAUCAAU
2721	GAAGAU C AUACGGU	2961	UUUUGU C AGCCACU
2724	AAGAUCA A CGGUUG	2962	UGUGAU C GUUCCAG
2744	GGUGGAG C CGUCAGG	2965	CUUGAAU C AAUAAAG
2750	GUCCUGU U UAAAAAC	2966	UGGAAGU C UUCAAGU
2759	GACGAAC A UCGAGUG	2969	GAUCAAU A AAGUUUA

2975	UGGAAGCU C UUCAAGCU
2976	UADAUGGU C CUCACCUG
2977	GAAGCUCU U CAAGCUGA

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Table 10: Rat ICAM HH Ribozyme Sequences

nt. Position	Rat HH Ribozyme Sequence		
11	UCAGUGUG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGAUC
23	UAGAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCAGC
26	AAGAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGUUC
31	AGGACCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAGG
34	GUUAUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCAG
40	GGGGCUUG	CUGAUGAGGCCGAAAGGCCGAA	ACCUUGAG
48	CCCAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCUC
54	GGCUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCGGGG
58	GGGAGCTA	CUGAUGAGGCCGAAAGGCCGAA	AGGCACGG
64	ACGGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUUG
96	AGGACCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAGG
102	GGCACCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAG
108	AGUUCCCC	CUGAUGAGGCCGAAAGGCCGAA	AGCAGUCC
115	UGGGACA	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGGA
119	GAGUUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUGUC
120	GGCCCGGG	CUGAUGAGGCCGAAAGGCCGAA	AUCACAAC
146	GGAGUCC	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCGG
152	UUGAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCGGGU
158	UGACUCGU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAAU
165	GGGGGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACUGUACA
168	CGAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUUC
185	CCUGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUCCACCC
209	GGUCAGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCTG
227	UUCACAGU	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGUC
230	CCUCCAC	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUUG
237	GGGGUGUC	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCAG
248	UCCUAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGGCC
253	CCUCCACU	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGUG
263	GCAUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCUC
267	CGAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUUC
293	UCAGCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAGCUUC
319	UCGUUUGU	CUGAUGAGGCCGAAAGGCCGAA	AUCCUCCG
335	AGUUCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCACAGU
337	GAGGACCA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCACA
338	CUCAGCUU	CUGAUGAGGCCGAAAGGCCGAA	AAGAGCUU
359	AAGCCGAG	CUGAUGAGGCCGAAAGGCCGAA	ACUGGUG
367	ACGGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUUG
374	AGGUGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUAA
375	GAGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUCU
378	UACCCUGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGGU
386	AGCUCCAA	CUGAUGAGGCCGAAAGGCCGAA	ACACAGCG

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394	CUGUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCACCCAC
420	UGGCGUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUGC
425	GGUGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCGAGG
427	UGGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACAGGGA
450	CGCAGGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCUU
451	GCCUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUACCC
456	UGGUGGCA	CUGAUGAGGCCGAAAGGCCGAA	AAGCCGAG
495	UACACAGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGGC
510	UCCCCACG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCAC
564	GUGGUUGG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUUC
592	UCCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AUACUCCC
607	GCAUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCUU
608	AGCAUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGCU
609	AAGCAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGGC
611	UGAAGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUUG
656	CAUUCUUG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUGAC
657	ACAUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AACAGUGA
668	AAGAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGUUC
677	UGGCGUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUGC
684	AAAGUCCG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCCU
692	GGAGUCC	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUGG
693	GGAAGAUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCCG
696	AGAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGGC
709	GUGAGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAADGCUU
720	GAGCUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUAG
723	UGGAGCUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAGUUG
735	GCGACCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAG
738	UCCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGGA
765	AGUUCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCAAGAU
769	UCCAGGG	CUGAUGAGGCCGAAAGGCCGAA	ACACAAGA
770	CUUCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACACAAG
785	AGGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCCU
786	GAGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGGCC
792	GCGACCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAG
794	GAGCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGGA
807	UCCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAGC
833	UAGUCUCC	CUGAUGAGGCCGAAAGGCCGAA	ACCCCAGG
846	CAAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	ACUGUCAG
851	AGCUGCUA	CUGAUGAGGCCGAAAGGCCGAA	AGGUGAGC
863	AOGGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUUG
866	UGUCAGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGCAUGG
867	UAGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGUC
869	CUUCGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGAG
881	CACGGGUU	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAUU
885	UUCACAGU	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGUC
933	CUGGGAAC	CUGAUGAGGCCGAAAGGCCGAA	AAUACACA
936	UGACACAA	CUGAUGAGGCCGAAAGGCCGAA	AUCUCUGC
978	AAGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	AUUCUCAA
980	AAAAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCUC

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986	GAGCUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUAG
987	GGAGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUUGUA
988	GGGAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUUGU
1005	GACGCCAC	CUGAUGAGGCCGAAAGGCCGAA	AUCACGAA
1006	CCUGGUGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCAC
1023	CCUUCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCGG
1025	CCCCUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGACCUCC
1066	UUGGGAAC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAGG
1092	UCUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AACCCUCU
1093	AGGGGCTG	CUGAUGAGGCCGAAAGGCCGAA	AUUCOCCT
1125	AUCAACAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGGG
1163	AGCAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGCGUCGU
1164	GAGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCGUCG
1166	CAGAGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCGU
1172	AGGCCGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAAG
1200	UUCAGUGU	CUGAUGAGGCCGAAAGGCCGAA	AUUGGAU
1201	CCUGUGGA	CUGAUGAGGCCGAAAGGCCGAA	AAGCCCAA
1203	GACCUUGG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCCC
1227	AGCACAUG	CUGAUGAGGCCGAAAGGCCGAA	AGUUCCAA
1228	ACGAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGCCCGC
1233	GCGACAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAG
1238	GAGGACCA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCACA
1264	ACCCGUUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUCCC
1267	CADUCUUG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUGAC
1294	CTGACACA	CUGAUGAGGCCGAAAGGCCGAA	AUUCUCUG
1295	UCUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCCUCUC
1306	GCAUGUAA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUGCU
1321	UUUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUGUU
1334	GCUCUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACGAUATC
1344	GGAUACCU	CUGAUGAGGCCGAAAGGCCGAA	AGCACCGA
1351	AGUCCUCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCUCGA
1353	CCAUGGUU	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCUA
1366	CCUGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUAOCCT
1367	GOCUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUACCC
1368	GGCAGCGG	CUGAUGAGGCCGAAAGGCCGAA	ACACCAUC
1380	ACCAUCCC	CUGAUGAGGCCGAAAGGCCGAA	AUAGGCAG
1388	CAUCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCCA
1398	UGUCCUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGCCAG
1402	CAGUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AAGCACAG
1408	GACGCCAC	CUGAUGAGGCCGAAAGGCCGAA	AUCACGAA
1410	GUCCACUC	CUGAUGAGGCCGAAAGGCCGAA	AUAGUUCG
1421	GCCUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUACCC
1425	AGCCAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGGU
1429	CCUGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGUUA
1444	CUCCUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUUCU
1455	UCCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AUACUCCC
1482	CCUGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUACCCU
1484	GCAAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGCAGU
1493	UAGUCUCC	CUGAUGAGGCCGAAAGGCCGAA	ACCCACGG

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1500	UUGA'CCAU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCACG
1503	GUGGUUGG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUUC
1506	CCAACAAU	CUGAUGAGGCCGAAAGGCCGAA	AUGACCCA
1509	UACACAGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGGC
1518	ACAACGGC	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAC
1530	ACAATUAT	CUGAUGAGGCCGAAAGGCCGAA	ACCCAGGU
1533	AAGCCCGC	CUGAUGAGGCCGAAAGGCCGAA	AUGAUCAG
1551	UACGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGGGCCAC
1559	UAAACAGG	CUGAUGAGGCCGAAAGGCCGAA	ACUUCCCA
1563	UGGGAACA	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGEA
1565	GGCGUAA	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUAA
1567	CUGGCGGU	CUGAUGAGGCCGAAAGGCCGAA	AUAGGUGU
1584	UAUAUCCU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUCUU
1592	GUAACUUG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCCTG
1599	GCCUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AACUUGUA
1651	GGCUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCGGGG
1661	ACCAGGGC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGCAG
1663	U'GUCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGUUC
1678	CCCAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCUC
1680	GACCUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCCC
1681	GAGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGGCC
1684	GAGAGGUC	CUGAUGAGGCCGAAAGGCCGAA	ACGAGCAG
1690	AAUGUADG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGGG
1691	GAAGAUOG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCGG
1696	GCGACCCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAG
1698	UCUCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCUGA
1737	GCACCGUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUGAUC
1750	AAUAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGGAC
1756	AGGACCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAGG
1787	CCCAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCUC
1790	GAGUUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUGUC
1793	GUCCAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGA'CCAU
1797	UGGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACAGGGA
1802	UCCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAGC
1812	UUUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUGUU
1813	ACGAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGCCCGC
1825	UACACAGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGGC
1837	UACCCUGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGGU
1845	GCCCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUCU
1856	GCAGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUAGGGG
1861	GGACCAUA	CUGAUGAGGCCGAAAGGCCGAA	AGCACADG
1865	CUUGUGUC	CUGAUGAGGCCGAAAGGCCGAA	ACCGGAUA
1868	AUUUAUAT	CUGAUGAGGCCGAAAGGCCGAA	ACUCGUGA
1877	CCUGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUACUGU
1901	UGUACCUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUAG
1912	UGUCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGUUC
1922	UAGGCAAU	CUGAUGAGGCCGAAAGGCCGAA	ACUUA'CAU
1923	CUAAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGCGUCCA
1928	UCCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAGC

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1930	CAUCCAGU	CUGAUGAGGCGAAAGGCCGAA	AGUCUCCA
1964	GCUGACAC	CUGAUGAGGCGAAAGGCCGAA	AAAUUCUC
1983	CCCAGGCC	CUGAUGAGGCGAAAGGCCGAA	AGGUUCUC
1996	AGCUUGAA	CUGAUGAGGCGAAAGGCCGAA	AGCUUCCA
2005	UAGGCAAU	CUGAUGAGGCGAAAGGCCGAA	ACUUAUAI
2013	CAUCCCGA	CUGAUGAGGCGAAAGGCCGAA	AGGCAGCG
2015	ACCAUCCC	CUGAUGAGGCGAAAGGCCGAA	AUAGGCAG
2020	GUACAGGG	CUGAUGAGGCGAAAGGCCGAA	ACUCAUAU
2039	UCGUUUGU	CUGAUGAGGCGAAAGGCCGAA	AUCCUCCG
2040	ACCUCCAG	CUGAUGAGGCGAAAGGCCGAA	AGGUCCAG
2057	AGCCAUUG	CUGAUGAGGCGAAAGGCCGAA	AGGACCAG
2061	UAGGUGUA	CUGAUGAGGCGAAAGGCCGAA	AUGGACGC
2071	CCUGAGGC	CUGAUGAGGCGAAAGGCCGAA	ACAAGUAU
2076	UUAGGCCU	CUGAUGAGGCGAAAGGCCGAA	AGGUUACA
2097	ACAUCAAC	CUGAUGAGGCGAAAGGCCGAA	AGAGUUGG
2098	ACCUCCAG	CUGAUGAGGCGAAAGGCCGAA	AGGUCCAG
2115	CAGGACCC	CUGAUGAGGCGAAAGGCCGAA	AGUCCGAA
2128	GAUCAUGG	CUGAUGAGGCGAAAGGCCGAA	ACAGCAU
2130	AGAGGCAG	CUGAUGAGGCGAAAGGCCGAA	AAACAGGC
2145	ACAUCAAC	CUGAUGAGGCGAAAGGCCGAA	AGAGUUGG
2152	AAGUUGUA	CUGAUGAGGCGAAAGGCCGAA	AUUCUCAA
2156	UCAUAAA	CUGAUGAGGCGAAAGGCCGAA	AACUGUCA
2158	AAUUAUAU	CUGAUGAGGCGAAAGGCCGAA	AUACAUCA
2159	GAUUAUAI	CUGAUGAGGCGAAAGGCCGAA	AAUACAUC
2160	UGAAUUAU	CUGAUGAGGCGAAAGGCCGAA	AAAUACAU
2162	AACAAAGG	CUGAUGAGGCGAAAGGCCGAA	AGGAUUGU
2163	CUCUGAAU	CUGAUGAGGCGAAAGGCCGAA	AAUUAUAU
2166	AAUUAUAU	CUGAUGAGGCGAAAGGCCGAA	AUACAUCA
2167	GAUUAUAI	CUGAUGAGGCGAAAGGCCGAA	AAUACAUC
2170	UCUGAAUU	CUGAUGAGGCGAAAGGCCGAA	AUAAAUAC
2171	UACUCAAU	CUGAUGAGGCGAAAGGCCGAA	AAUACUCG
2173	GAGGACCA	CUGAUGAGGCGAAAGGCCGAA	AUAGCACA
2174	AGCAGGGG	CUGAUGAGGCGAAAGGCCGAA	AAUAGAGA
2175	UEACUCGU	CUGAUGAGGCGAAAGGCCGAA	AAAGAAAU
2176	GUGGUUGG	CUGAUGAGGCGAAAGGCCGAA	ACAUUUUC
2183	UCAAUAAA	CUGAUGAGGCGAAAGGCCGAA	AACUGUCA
2185	ACUCAUAU	CUGAUGAGGCGAAAGGCCGAA	AUACUGU
2186	UACUCAAU	CUGAUGAGGCGAAAGGCCGAA	AAUACUCG
2187	GUACUCAA	CUGAUGAGGCGAAAGGCCGAA	AAAUAACT
2189	GGGUACUC	CUGAUGAGGCGAAAGGCCGAA	AUAAAUAA
2196	CAAUAAAU	CUGAUGAGGCGAAAGGCCGAA	ACUGUCAG
2198	UGACCUUG	CUGAUGAGGCGAAAGGCCGAA	AGACAUUC
2199	CUGGCAUG	CUGAUGAGGCGAAAGGCCGAA	AAGAGUCU
2200	GCCUGGGG	CUGAUGAGGCGAAAGGCCGAA	AAGUACCC
2201	GACCUUGG	CUGAUGAGGCGAAAGGCCGAA	AGAAGCCC
2205	CAGUGGCU	CUGAUGAGGCGAAAGGCCGAA	ACACAAAA
2210	CAUCCAGU	CUGAUGAGGCGAAAGGCCGAA	AGUCUCCA
2220	CCCAGGCC	CUGAUGAGGCGAAAGGCCGAA	AGGUUCUC
2224	AAGGUAGG	CUGAUGAGGCGAAAGGCCGAA	AUGUAUGU

2226	UGUGGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCAG
2233	AGUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGCAUGA
2242	ACUACUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGUGU
2248	GCGACCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAG
2254	UUCAGUGU	CUGAUGAGGCCGAAAGGCCGAA	AADUGGAU
2259	GCACCGUG	CUGAUGAGGCCGAAAGGCCGAA	ADGUGAUC
2260	AGCACCGU	CUGAUGAGGCCGAAAGGCCGAA	AADUGGAU
2266	AACUUGUA	CUGAUGAGGCCGAAAGGCCGAA	ADCCUGAU
2274	UACAUGUU	CUGAUGAGGCCGAAAGGCCGAA	ACCUGCUC
2279	ACCCGUAU	CUGAUGAGGCCGAAAGGCCGAA	ADCUUUCU
2282	ACUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAACUGU
2288	CAUUGGAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGGC
2291	GUAACUUG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCCUG
2321	ACCCGUAU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUCU
2338	CCUGUGGA	CUGAUGAGGCCGAAAGGCCGAA	AAGCCCAA
2339	GCCUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUACCC
2341	UGAGCACC	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCCC
2344	GAGAGGUC	CUGAUGAGGCCGAAAGGCCGAA	ACGAGCAG
2358	UGUGGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGGG
2359	UUCUGUGG	CUGAUGAGGCCGAAAGGCCGAA	ADGGAUGG
2360	CUUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACACAAG
2376	AAGAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGUUC
2377	UAUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGUC
2378	UCGUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACGC
2379	CGCAAGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAGCAG
2380	ACUCGUGA	CUGAUGAGGCCGAAAGGCCGAA	AGAAADCA
2382	UGACUCGU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAAU
2384	CUUGUGUC	CUGAUGAGGCCGAAAGGCCGAA	ACCGGAUA
2399	CGUCCACA	CUGAUGAGGCCGAAAGGCCGAA	AGUAUUA
2401	GAGGACCA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCACA
2411	UGAAGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUUG
2417	AACUUGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGAU
2418	AGUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGCAUGA
2425	GAACUCUG	CUGAUGAGGCCGAAAGGCCGAA	AUUAADUA
2426	UAGUCUCC	CUGAUGAGGCCGAAAGGCCGAA	ACCCAGG
2433	AACUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUGA
2434	UCGUUGU	CUGAUGAGGCCGAAAGGCCGAA	AUCCUCUG
2448	GGGGGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACUGUUA
2449	CGAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUUC
2451	GAGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGGCC
2452	AGAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGGC
2455	AACAAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGAADGU
2459	UGUGGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGGG
2460	UUGGGAAC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAGG
2479	GGCGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUA
2480	GGGAUCAC	CUGAUGAGGCCGAAAGGCCGAA	ACGGCGAC
2483	ACAUGGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAAAGGU
2484	GACAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AACAAAGG
2492	UAGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGUC

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2504	UAGGAADG	CUGAUGAGGCCGAAAGGCCGAA	AUGUAGGU
2508	AAGGUAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGUADGU
2509	AAAGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAUGUADG
2510	AAUAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGGAC
2520	ACAUUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAAAGGU
2521	GACAUUGG	CUGAUGAGGCCGAAAGGCCGAA	AACAAAGG
2533	UGAGGGGU	CUGAUGAGGCCGAAAGGCCGAA	AAUGCUGU
2540	GGAUACCU	CUGAUGAGGCCGAAAGGCCGAA	AGCACCGA
2545	AAAGUCCG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCCU
2568	CUGACACA	CUGAUGAGGCCGAAAGGCCGAA	AAUCUCUG
2579	CCAGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGCAGG
2585	GAGAGGUC	CUGAUGAGGCCGAAAGGCCGAA	ACGAGCAG
2588	GGCUGUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGCA
2591	CUUGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGAG
2593	AGCAGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAGA
2596	GOGACCA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAG
2601	GAGGACCA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCACA
2602	ACAACGGC	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAC
2607	CCUGGUGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCAC
2608	UCCACCG	CUGAUGAGGCCGAAAGGCCGAA	AGCUAAAG
2609	CAUCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCCA
2620	AACUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUGA
2626	AGCAGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACUGAGAG
2628	GGAGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUUGUA
2635	GUGAAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGUGA
2640	UGGAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUGAGC
2641	AAUGUADG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGGG
2642	AGAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGGC
2653	AGCACCCU	CUGAUGAGGCCGAAAGGCCGAA	ACCUGUGG
2659	GCUUGCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCCUUCU
2689	AGCUUCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCCUAGU
2691	AGUCCUCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUGA
2700	CCUGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUACCCU
2704	UAGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGUC
2711	ACCUUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGGG
2712	CACCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAGG
2721	ACCCGUAT	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUCC
2724	CAAACCOG	CUGAUGAGGCCGAAAGGCCGAA	AUGAUCUU
2744	CCUGCACG	CUGAUGAGGCCGAAAGGCCGAA	AUCCACCC
2750	GGUUUUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGGAC
2759	CCACUUGA	CUGAUGAGGCCGAAAGGCCGAA	AGUUCGUC
2761	GGAAGAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCCG
2765	AGGCCGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAAG
2769	GCAGGGGU	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGAA
2797	UUGACCAU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCACG
2803	GUUCUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAUGAG
2804	AGUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGCAUGA
2813	AGGGUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGGGAGC
2815	GGAAGAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCCG

2821	ACCUCCAG	CUGAUGAGGCGAAAGGCCGAA	AGGUCAGG
2822	GGAGCUGA	CUGAUGAGGCGAAAGGCCGAA	AAGUUGUA
2823	UGGGAGCU	CUGAUGAGGCGAAAGGCCGAA	AAAAGUUG
2829	GGAUACCU	CUGAUGAGGCGAAAGGCCGAA	AGCACCGA
2837	GGGGGAAG	CUGAUGAGGCGAAAGGCCGAA	ACCUCUGG
2840	UGCGCUGG	CUGAUGAGGCGAAAGGCCGAA	AGGGGUGC
2847	AGGUGGGU	CUGAUGAGGCGAAAGGCCGAA	AGGGGUAA
2853	CUAGUCGG	CUGAUGAGGCGAAAGGCCGAA	AGAUUGAA
2860	UUCGAGGG	CUGAUGAGGCGAAAGGCCGAA	ACACAAGA
2872	UGAGCACC	CUGAUGAGGCGAAAGGCCGAA	ACAGGCCC
2877	GGUGCUGG	CUGAUGAGGCGAAAGGCCGAA	AGACUCCA
2899	AAAGUCGG	CUGAUGAGGCGAAAGGCCGAA	AGCUGCCU
2900	AGAGAAGG	CUGAUGAGGCGAAAGGCCGAA	AGUCAGCC
2904	AAGAGGAA	CUGAUGAGGCGAAAGGCCGAA	AGCAGUUC
2905	AGAGAAGG	CUGAUGAGGCGAAAGGCCGAA	AGUCAGCC
2906	UUAUAUAA	CUGAUGAGGCGAAAGGCCGAA	ACAUCAAC
2907	CGCAAGAG	CUGAUGAGGCGAAAGGCCGAA	AAGAGCAG
2908	AADUAUAU	CUGAUGAGGCGAAAGGCCGAA	ADACADCA
2909	AAGAGGAA	CUGAUGAGGCGAAAGGCCGAA	AGCAGUUC
2910	GUAAUAGA	CUGAUGAGGCGAAAGGCCGAA	AAGGAAGU
2911	GGGUAAUA	CUGAUGAGGCGAAAGGCCGAA	AGAAGGAA
2912	UGAAUUAU	CUGAUGAGGCGAAAGGCCGAA	AAAUACAU
2913	CUGGGAAC	CUGAUGAGGCGAAAGGCCGAA	AUAACACA
2914	UCUGAAUU	CUGAUGAGGCGAAAGGCCGAA	AUAADUAC
2915	CUCUGAAU	CUGAUGAGGCGAAAGGCCGAA	AAUAUAUA
2916	CUUCGCAA	CUGAUGAGGCGAAAGGCCGAA	AGGAAGAG
2917	GUCUUCGC	CUGAUGAGGCGAAAGGCCGAA	AGAGGAAG
2918	UGACUCGU	CUGAUGAGGCGAAAGGCCGAA	AAAGAAAU
2919	CAGUGGCU	CUGAUGAGGCGAAAGGCCGAA	ACACAAAA
2931	GGCAGCGG	CUGAUGAGGCGAAAGGCCGAA	ACACCAUC
2933	GGUGCUGG	CUGAUGAGGCGAAAGGCCGAA	AGACUCCA
2941	GCCUGGGG	CUGAUGAGGCGAAAGGCCGAA	AAGUACUG
2951	GUCAGAGG	CUGAUGAGGCGAAAGGCCGAA	AGCAUGGU
2952	GAAGAUCG	CUGAUGAGGCGAAAGGCCGAA	AAGUCCGG
2955	CCAUGUCA	CUGAUGAGGCGAAAGGCCGAA	AGGAAGCA
2956	AUUGAUUC	CUGAUGAGGCGAAAGGCCGAA	AAGGAAGG
2961	CAGUGGCU	CUGAUGAGGCGAAAGGCCGAA	ACACAAAA
2962	CUGGGAAC	CUGAUGAGGCGAAAGGCCGAA	AAUACACA
2965	ACUUUAUU	CUGAUGAGGCGAAAGGCCGAA	AUUCAAAG
2966	AGCUUGAA	CUGAUGAGGCGAAAGGCCGAA	AGCUUCCA
2969	UAAAACUU	CUGAUGAGGCGAAAGGCCGAA	AUUGAUUC
2975	AGCUUGAA	CUGAUGAGGCGAAAGGCCGAA	AGCUUCCA
2976	CAGGUGAG	CUGAUGAGGCGAAAGGCCGAA	ACCAUUAU
2977	UCAGCUUG	CUGAUGAGGCGAAAGGCCGAA	AGAGCUUC

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Table 11: Human IL-5 HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	AUGCACU U UCUUUGC	245	AAGAAAU C UUUCAGG
9	UGCACUU U CUUUGCC	247	GAAAUUU U UCAGGGA
10	GCACUUU C UUUGCCA	248	AAAUUUU U CAGGGGA
12	ACUUCUU U UGCCAAA	249	AADUUUU C AGGGAAU
13	CUUUCUU U GCCAAAG	257	AGGGAAU A GGCACAC
36	AGAACGU U UCAGAGC	273	GGAGAGU C AAACUGU
37	GAACGUU U CAGAGCC	291	AGGGGGU A CUGUGGA
38	AACGUUU C AGAGCCA	305	AAAGACU A UUCAAAA
56	GGAUGCU U CUGCAUU	307	AGACUAA U CAAAAAC
57	GAUGCUU C UGCAUUU	308	GACUAAU C AAAAACT
63	UCUGCAU U UGAGUUU	316	AAAAACT U GUCCUUA
64	CUGCAUU U GAGUUUG	319	AACUUGU C CUUAAUA
69	UUUGAGU U UGCUAGC	322	UUGUCCU U AAUAAAG
70	UUGAGUU U GCUAGCU	323	UGUCCUU A AUAAAGA
74	GUUUGCU A GCUUUG	326	CCUAAAU A AAGAAAU
78	GUAGCUU C UUGGAGC	334	AAGAAAU A CAUUGAC
80	UAGCUUU U GGAGCUG	338	AUUACAU U GACGGCC
91	GCUGCCU A CGUGUAU	380	GGAGAGU A AACCAAU
97	UACGUGU A UGCCADC	388	AACCAAU U CCUAGAC
104	AUGCCAU C CCCACAG	389	ACCAAUU C CUAGACU
116	CAGAAAU U CCCACAA	392	AAUUCUU A GACTUAC
117	AGAAAUU C CCACAAG	397	CUAGACU A CCUGCAA
130	AGUGCAU U GGUGAAA	409	CAAGAGU U UCUUGGU
145	GAGACCU U GGCACUG	410	AAGAGUU U CUUGGGU
155	CACUGCU U UCUACUC	411	AGAGUUU C UUGGUGU
156	ACUGCUU U CUACUCA	413	AGUUUCU U GGUGUAA
157	CUGCUUU C UACUCAU	419	UUGGUGU A AUGAACA
159	GUUUUUU A CUCAUCG	437	AGUGGAU A AUAGAAA
162	UUUUAU C AUUGAAC	440	GGAUAAU A GAAAGUU
165	UACUCAU C GAACUCU	447	AGAAAGU U GAGACTA
171	UCGAACU C UGUGAUU	454	UGAGACU A AACUGGU
179	UGCUGAU A GCCAADG	462	AACUGGU U UGUUGCA
192	UGAGACU C UGAGGAU	463	ACUGGUU U GUUGCAG
200	UGAGGAU U CCUGUUC	466	GGUUUGU U GCAGCCA
201	GAGGAUU C CUGUCC	479	CAAAGAU U UUGGAGG
206	UCCUGU U CCUGUAC	480	AAAGAUU U UGGAGGA
207	UCCUGUU C CUGUACA	481	AAGAUUU U GGAGGAG
212	UCCUGU A CAUAAAA	497	AGGACAU U UACUGC
216	UGUACAU A AAAADCA	498	GGACAUU U UACUGCA
222	UAAAAAU C ACCAACU	499	GACAUUU U ACUGCAG

500	ACAUUUU A CUGCAGU	684	UACUUUU U UCUUADU
531	AAAGAGU C AGGCCUU	685	ACUUUUU U CUUADUU
538	CAGGCCU U AAUUUUC	686	CUUUUUU C UUAUUUA
539	AGGCCUU A ADUUUCA	688	UUUUUCU U AUUUUAC
542	CCUUAUU U UUCAUAU	689	UUUUUCU A UUUAACU
543	CUUAUUU U UCAUAUU	691	UUCUUUU U UAACUUA
544	UUAUUUU U CAUAUAU	692	UCUUUUU U AACUUAA
545	UAAUUUU C AAUAUAU	693	CUUAUUU A ACUUUAC
549	UUUCAUU A UAUAUAU	697	UUUAACU U AACAUUC
551	UCAUAUU A AUUUUAC	698	UUAACUU A ACAUUUC
554	AUAUAUU U UAACUUC	703	UUAACUU U CUGUAAA
555	UAUAUUU U AACUCCA	704	UAACUUU C UGUAAAA
556	AUAUUUU A ACUUCAG	708	ADUCUGU A AAADGUC
560	UUUAACU U CAGAGGG	715	AAAADGU C UGUUAAC
561	UUUAACU C AGAGGGA	719	UGUCUGU U AACUUAA
573	GGAAAGU A AAUAUUU	720	GUCUGUU A ACUUAAU
577	AGUAAAU A UUUCAGG	724	GUUAACU U AAUAUGU
579	UAAUAUU U UCAGGCA	725	UUAACUU A ADAGUAU
580	AAUAUUU U CAGGCAU	728	ACUUAUU A GUUUUUA
581	AAUAUUU C AGGCAUA	731	UAUAUGU A UUUAUGA
588	CAGGCAU A CUGACAC	733	AUAUGUU U UAUGAAA
597	UGACACU U UGCCAGA	734	UAGUAUU U ADGAAAU
598	GACACUU U GCCAGAA	735	AGUAUUU A UGAAADG
611	AAAGCAU A AAUUUCU	745	AAAUUGU U AAGAUUU
616	AUAUUUU U CUUAAAA	746	AAUGGUU A AGAUUUU
617	UAAAAUU C UUUAAAU	752	UAAGAAU U UGGUAAA
619	AAAUUCU U AAAUAUU	753	AAGAAUU U GGUAAAA
620	AAUUCUU A AAUAUAU	757	AUUUGGU A AAUAUGU
625	UUAAAAU A UAUUUCA	761	GGUAAAU U AGUAUUU
627	AAAAUAU A UUUCAGA	762	GUAAAUU A GUUUUUA
629	AAUAUUU U UCAGAUU	765	AAUAUGU A UUUAUUU
630	AUAUAUU U CAGAUUU	767	UUAGUAU U UAUUUAA
631	UAUAUUU C AGAUUUC	768	UAGUAUU U AUUUAAU
636	UUCAGAU A UCAGAAU	769	AGUAUUU A UUUAADG
638	CAGAUUU C AGAAUCA	771	UAUUUAU U UAADGUU
644	UCAGAAU C ADUGAAG	772	AUUUAUU U AADGUUA
647	GAAUCAA U GAAGUAU	773	UUUAUUU A ADGUUAU
653	UUGAAGU A UUUUCCU	778	UUUAADG U ADGUUGU
655	GAAGUAU U UCCUCC	779	UAADGUU A UGUUGUG
656	AAGUAUU U UCCUCCA	783	GUUAUGU U GUGUUCU
657	AGUAUUU U CCUCCAG	788	GUUGUGU U CUAAUAU
658	GUUUUUU C CUCCAGG	789	UUGUGUU C UAUAUAU
661	UUUUCCU C CAGGCAA	791	GUGUUCU A AUAAAAAC
672	GCAAAAU U GAUAUAC	794	UUCUAUU A AAACAAA
676	AAUUGAU A UACUUUU	805	CAAAAAU A GACAACU
678	UUGAUUU A CUUUUUU		
581	AUAUAUU U UUUUCUU		
682	UAUAUUU U UUUCUUA		

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Table 12: Human IL-5 HH Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
8	GCAAAGA CUGAUGAGGCCGAAAGGCCGAA AGUGCAU
9	GGCAAAG CUGAUGAGGCCGAAAGGCCGAA AAGUGCA
10	UGGCAAA CUGAUGAGGCCGAAAGGCCGAA AAAGUGC
12	UUUGGCA CUGAUGAGGCCGAAAGGCCGAA AGAAAGU
13	CUUUGGC CUGAUGAGGCCGAAAGGCCGAA AAGAAAG
36	GCUCUGA CUGAUGAGGCCGAAAGGCCGAA ACGUUCU
37	GGCUCUG CUGAUGAGGCCGAAAGGCCGAA AACGUUC
38	UGGCUCU CUGAUGAGGCCGAAAGGCCGAA AAACGUU
56	AAUGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAUCC
57	AAAUCCA CUGAUGAGGCCGAAAGGCCGAA AAGCAUC
63	AAACUCA CUGAUGAGGCCGAAAGGCCGAA AUGCAGA
64	CAAACUC CUGAUGAGGCCGAAAGGCCGAA AAUGCAG
69	GCUAGCA CUGAUGAGGCCGAAAGGCCGAA ACUCAAA
70	AGCUAGC CUGAUGAGGCCGAAAGGCCGAA AACUCAA
74	CAAGAGC CUGAUGAGGCCGAAAGGCCGAA AGCAAAC
78	GCUCCAA CUGAUGAGGCCGAAAGGCCGAA AGCUAGC
80	CAGCUCC CUGAUGAGGCCGAAAGGCCGAA AGAGCUA
91	AUACACG CUGAUGAGGCCGAAAGGCCGAA AGGCAGC
97	GADUGGA CUGAUGAGGCCGAAAGGCCGAA ACACGUA
104	CUGUGGG CUGAUGAGGCCGAAAGGCCGAA ADGGCAU
116	UUGUGGG CUGAUGAGGCCGAAAGGCCGAA ADUUCUG
117	CUUGUGG CUGAUGAGGCCGAAAGGCCGAA ADUUCU
130	UUUCACC CUGAUGAGGCCGAAAGGCCGAA AUGCACU
145	CAGUGCC CUGAUGAGGCCGAAAGGCCGAA AGGUCUC
155	GAGUAGA CUGAUGAGGCCGAAAGGCCGAA AGCAGUG
156	UGAGUAG CUGAUGAGGCCGAAAGGCCGAA AAGCAGU
157	AUGAGUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAG
159	CGAUGAG CUGAUGAGGCCGAAAGGCCGAA AGAAAGC
162	GUUCGAU CUGAUGAGGCCGAAAGGCCGAA AGUAGAA
165	AGAGUUC CUGAUGAGGCCGAAAGGCCGAA AUGAGUA
171	AUCAGCA CUGAUGAGGCCGAAAGGCCGAA AGUUGCA
179	CAUUGGC CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
192	AUCCUCA CUGAUGAGGCCGAAAGGCCGAA AGUCUCA
200	GAACAGG CUGAUGAGGCCGAAAGGCCGAA AUCCUCA
201	GGAACAG CUGAUGAGGCCGAAAGGCCGAA AUCCUC
206	GUACAGG CUGAUGAGGCCGAAAGGCCGAA ACAGGAA
207	UGUACAG CUGAUGAGGCCGAAAGGCCGAA AACAGGA
212	UUUUAUG CUGAUGAGGCCGAAAGGCCGAA ACAGGAA
216	UGAUUUU CUGAUGAGGCCGAAAGGCCGAA AUGUACA
222	AGUUGGU CUGAUGAGGCCGAAAGGCCGAA AUUUUUA
245	CCUGAAA CUGAUGAGGCCGAAAGGCCGAA AUUUCUU

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247	UCCUCGA	CUGAUGAGGCCGAAAGGCCGAA	AGAUUUC
248	UCCUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGAUUU
249	AUUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUU
257	GUGUGCC	CUGAUGAGGCCGAAAGGCCGAA	AUUCUUU
273	ACAGUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCC
291	UCCACAG	CUGAUGAGGCCGAAAGGCCGAA	ACCCUUU
305	UUUUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUUU
307	GUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUAGUCU
308	AGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGUC
316	UAAGGAC	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUU
319	UAUAUAG	CUGAUGAGGCCGAAAGGCCGAA	ACAAGUU
322	CUUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AGGACAA
323	UCUUUAU	CUGAUGAGGCCGAAAGGCCGAA	AAGGACA
326	AUUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AUUAAGG
334	GUCAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUU
338	GGCCGUC	CUGAUGAGGCCGAAAGGCCGAA	AUGUAUU
380	AUUGGUU	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCC
388	GUCUAGG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGUU
389	AGUCUAG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGU
392	GGUAGUC	CUGAUGAGGCCGAAAGGCCGAA	AGGAUUU
397	UUGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUAG
409	ACCAAGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUG
410	CACCAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUCUUU
411	ACACCAA	CUGAUGAGGCCGAAAGGCCGAA	AAACUUU
413	UUACACC	CUGAUGAGGCCGAAAGGCCGAA	AGAAACU
419	UGUUCAU	CUGAUGAGGCCGAAAGGCCGAA	ACACCAA
437	UUUCUAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCACU
440	AACUUUC	CUGAUGAGGCCGAAAGGCCGAA	AUUAUCC
447	UAGUCUC	CUGAUGAGGCCGAAAGGCCGAA	ACUUUCU
454	ACCAGUU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCA
462	UGCAACA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGUU
463	CUGCAAC	CUGAUGAGGCCGAAAGGCCGAA	AACCAGU
466	UGGCUGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAACC
479	CCUCCAA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUG
480	UCCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AAUCUUU
481	CUCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AAAUCUU
497	GCAGUAA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCCU
498	UGCAGUA	CUGAUGAGGCCGAAAGGCCGAA	AAUGUCC
499	CUGCAGU	CUGAUGAGGCCGAAAGGCCGAA	AAADGUC
500	ACUGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAUGU
531	AAGGCCU	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUU
538	GAAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUG
539	UGAAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCU
542	UAUUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUUAAGG
543	AUAUUGA	CUGAUGAGGCCGAAAGGCCGAA	AAUUAAG
544	UAUAUUG	CUGAUGAGGCCGAAAGGCCGAA	AAAUUAA
545	UUAUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAUUA
549	UAAAUUA	CUGAUGAGGCCGAAAGGCCGAA	AUUGAAA
551	GUUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUGA

554	GAAGUUA	CUGAUGAGGCCGAAAGGCCGAA	AUUAUAU
555	UGAAGUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUA
556	CUGAAGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUUAU
560	CCCUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGUUAUA
561	UCCUCUCU	CUGAUGAGGCCGAAAGGCCGAA	AAGUUAU
573	AAAUUAU	CUGAUGAGGCCGAAAGGCCGAA	ACUUUCC
577	CCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAU
579	UGCCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUAUUA
580	AUGCCUG	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
581	UAUGCCU	CUGAUGAGGCCGAAAGGCCGAA	AAAUUAU
588	GUGUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCUG
597	UCUGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGUCA
598	UUCUGGC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUC
611	AGAAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUU
616	UUUAAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUAU
617	AUUUUA	CUGAUGAGGCCGAAAGGCCGAA	AAUUUA
619	AUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUU
620	UAUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAGAAUU
625	UGAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAU
627	UCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AUAUUU
629	UAUCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAU
630	AUAUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAUAUAU
631	GADAUCU	CUGAUGAGGCCGAAAGGCCGAA	AAAUUA
636	AUUCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAA
638	UGAUUCU	CUGAUGAGGCCGAAAGGCCGAA	AUAUCUG
644	CUUCAAU	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
647	AUAUCUC	CUGAUGAGGCCGAAAGGCCGAA	AUGAUUC
653	AGGAAAA	CUGAUGAGGCCGAAAGGCCGAA	ACUUCAA
655	GGAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUAUCUC
656	UGGAGGA	CUGAUGAGGCCGAAAGGCCGAA	AAUAUCU
657	CUGGAGG	CUGAUGAGGCCGAAAGGCCGAA	AAAUUCU
658	CCUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AAAUUC
661	UUGCCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAAAA
672	GUUAUC	CUGAUGAGGCCGAAAGGCCGAA	AUUUUGC
676	AAAAGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCAAUU
678	AAAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCAA
681	AAGAAAA	CUGAUGAGGCCGAAAGGCCGAA	AGUAUAU
682	UAAGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGUAUA
683	AUAAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAAGUAU
684	AAUAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGUA
685	AAAUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAAGU
686	UAAAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAAAAG
688	GUUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAA
689	AGUUAUA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAAA
691	UAAGUUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAGAA
692	UUAAGUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAGA
693	GUUAAGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAG
697	GAAUGUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUAUA
698	AGAAUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGUUAU

703	UUUACAG	CUGAUGAGGCCGAAAGGCCGAA	AUGUUA
704	UUUUACA	CUGAUGAGGCCGAAAGGCCGAA	AAUGUUA
708	GACAUUU	CUGAUGAGGCCGAAAGGCCGAA	ACAGAAU
715	GUUAACA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUU
719	UUUAGUU	CUGAUGAGGCCGAAAGGCCGAA	ACAGACA
720	AUUUAGU	CUGAUGAGGCCGAAAGGCCGAA	AACAGAC
724	UACUAUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUAAC
725	AUACUAU	CUGAUGAGGCCGAAAGGCCGAA	AAGUUAA
728	UAAAUAC	CUGAUGAGGCCGAAAGGCCGAA	AUUUAGU
731	UCAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACTAUUA
733	UUUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AUACUAU
734	AUUUCAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACUA
735	CAUUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACT
745	AAUUCUU	CUGAUGAGGCCGAAAGGCCGAA	ACCAUUU
746	AAAUUCU	CUGAUGAGGCCGAAAGGCCGAA	AACCAUU
752	UUUACCA	CUGAUGAGGCCGAAAGGCCGAA	AUUCUUA
753	AUUUACC	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUU
757	ACUAAUU	CUGAUGAGGCCGAAAGGCCGAA	ACCAAUU
761	AAAUACT	CUGAUGAGGCCGAAAGGCCGAA	AUUUACC
762	UAAAUAC	CUGAUGAGGCCGAAAGGCCGAA	AAUUUAC
765	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACTAUUU
767	UUAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AUACUAA
768	AUUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACUA
769	CAUUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACT
771	AACAUUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUU
772	UAACAUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
773	AUAACAU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAA
778	ACAACAU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUAA
779	CACAACA	CUGAUGAGGCCGAAAGGCCGAA	AACAUUA
783	AGAACAC	CUGAUGAGGCCGAAAGGCCGAA	ACAUUAC
788	UUUUUAG	CUGAUGAGGCCGAAAGGCCGAA	ACACAAC
789	UUUAUUA	CUGAUGAGGCCGAAAGGCCGAA	AACACAA
791	GUUUUAU	CUGAUGAGGCCGAAAGGCCGAA	AGAACAC
794	UUUGUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUAGAA
805	AGUUGUC	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUG

Table 13: Mouse IL-5 HH Ribozyme Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	cGCUUUU c CUUUGCU	253	AGGGgcU A GaCAuAC
11	uCUUcCU U UGUGgAA	259	UagACAU a CUGaAgA
12	CUUcCUU U GCUgAAG	269	GaAGaAU C AAACUGU
36	GAAgacU U CAGAGuC	269	GaAGaAU c AAaCugU
36	GaAgAcU u cAgAGUc	269	GAAGAau c aAAcUgU
37	AAgacUU C AGAGuCA	287	uGGGGGU A CUGUGGA
43	UcaGaGU c ADGagaA	301	AAAuugCU A UUCcAAA
58	GGUUGCU U CUGCacU	301	AAAuugCU a uUCCaaa
59	GAUGCUU C UGCacUU	303	AUGCUAU u CCaAaAc
59	gAUGcUU c uGcAcUU	303	AugCUAU U CcAAAAC
66	CUGCacU U GAGUgUu	304	ugCUAUU C cAAAACc
82	UgAcucU c aGcUGUG	315	AAcCUGU C aUUAADA
91	GcUgUGU c uggGCCA	318	cUGUCaU U AAUAAG
112	ugGagAU U CCCAugA	319	UGUCaUU A AUAAAGA
113	gGagAUU C CCAugAG	322	CaUUAUU A AAGAAAU
141	GAGACCU U GaCACA	330	AAGAAAU A CAUGGAC
141	GAgACcU U GaCacAg	334	AAUACAU U GACcGCC
158	gUCcgCU C AcCGAgC	334	AAUaCaU u GACcgCC
167	cCGAgCU C UGUUGAc	384	AggCagU U CCUGGau
196	UGAGGcU U CCUGUcC	385	ggCagUU C CUGGauU
197	GAGGcUU C CUGUcCC	393	CUgGauU A CCUGCAA
197	gAGGCUU c CUGUcCC	405	CAAGAGU U cCUUGGU
202	UUCCUGU c CCUacUC	406	AAAGAGU c CUUGGUG
202	UUCCUGU c CcUAcuc	409	AGUUCcU U GGUGUGA
206	UGUCccU a cuCaUAA	481	UcaCAAU u UAAgUUA
212	UACUCAU a aAAaUCa	482	cAcAAAU U AAgUUaA
212	UacuCAU A AAAAUCA	483	AcAAUUU A AgUUaAa
218	UaaAaaU c aCCAGCU	483	AcAAUuU a aGUUAaA
218	UAAAAAU C ACCAgCU	495	AAAUUgU c AAcAgAU
218	uAAAAAU c accAgCU	553	GCUGuuU c CaUuUAU
232	uaUGCAU U GGaGAAA	557	UuUcCAU U UauaUUU
241	gAGAAAU C UUUCAGG	564	UUzuAuU u aUgUCCU
241	gAgAaAU c UUucAGG	564	UUauaUU u AugUcCU
241	gagAAAU c UUUCAGG	565	uaUAUUU a ugUCCUG
241	gAgAaAU c UUUCAGg	565	UAUAuuU a UgUCCUg
243	gaAAucU U UCAGgGg	569	UUuADGU c cUGUaGU
243	GAAAUcU U UCAGGGg	569	uUUUAUGU c cUGUagU
244	AAAUCUU U CAGGGgc	613	AAAGuGU u uaaCCUU
245	AAUCUUU C AGGGgcU	614	AAgUGuU u aACcUUU

620	UUAACcU u uUuGUAU	1407	cCAgUUU A CUcCAGg
793	caAGgCU u UGuGcAU	1407	ccAgUUU a CUCCAGG
816	CUGagUU a UACUCcc	1410	gUUUaCU C CAGGaAA
818	GAgUUAU a cUCCcuC	1434	ADgCUUU U aUuUaAU
825	ACUcCcU c CccCUCA	1434	aUgcUuU U AUUUAAu
825	aCUccCU c CcCcUCA	1434	aUgcUuU u AuUUAAU
839	AuCcucU U cGUUGCA	1435	UgCUUUU a UuUaAUU
840	uCcucUU c GUUGCAu	1435	ugcUUUU a uUUaAUU
863	cAAGUAU U cCAGGCU	1438	UuUUAAU U AAuUcug
864	AAgUAUU c CAGGCug	1438	uUUUAUU U AAUucUg
864	AAGUAUU c caggCug	1439	UUUAUUU A AUucUgU
913	gAaCUUU U GGuCaG	1443	UUUAuU c UGuAAGa
917	UcUgggU c CAGAuGG	1447	ADUCUGU A AgAUgUu
957	UUagcAU c CUUcUc	1458	ugUUcaU a UUAUUUA
960	GCAuccU u UcUcCUa	1458	ugUUcAU A uUAUUUA
960	GcaUcCU u cCUcCUa	1460	UucAAUU u AUUUAug
962	AUcCUuU c UCCUaGC	1461	UcAUaU A UUUUAUGA
975	gccccCU u AgAUAgA	1463	AUAUUUU U UADGAug
987	aGaUGAU A cuuAAUG	1475	AuGgAUU c aGUAAgU
990	UGAuACU u AAagacU	1479	AUUcaGU A AgUUAAU
1000	UGACuCU c UugCuGA	1483	aGuAAGU u AAUAUUU
1027	CgggGCU U cCUgCUC	1483	aGUAAgU U AaUAUUU
1034	UCCUGcU C CUaUcuA	1484	GUAAGUU A aUAUUUA
1037	UgcUcCU A UcUAACU	1487	agUUAAU a UUUuAUa
1039	cUccuAU c UAACUUC	1487	AgUUaAU A UUUUAUa
1039	cUCCUAU c UAACUUC	1489	UUAAUaU U uAuUAcA
1041	CcUAUcU A ACUUCaA	1489	UUAAuAU u UAUUaCA
1051	UUcAAuU U AAuAccc	1489	UUaAUU U UAUUaCA
1148	uGAcUUU u cUuaUGU	1490	UAAUaUU u AuUAcAc
1213	GCUGGaU u UUGGAaa	1490	UAaUAUU U ADuAcAc
1213	gcUGGAU u uUgGAAA	1490	UAaUAUU U ADUAcAc
1214	cugGAUU U UGGAAAa	1491	AAUAUUU a uuACAg
1215	ugGAUUU U GGAaaAG	1491	AAUAUUU a UuAcAcg
1234	gGGACAU c UccuUGC	1491	AaUAUUU A UuAcAcG
1236	GACAUcU c cuUGCAG	1491	AaUAUUU A UuAcAcG
1275	ugGGCCU U AcUUcUC	1494	AUUUAUU a CAgUAU
1276	gGGCCUU A cUUcUCC	1502	cACGUaU A UaauAUu
1280	CUUAcUU c UCcgUgU	1502	cAcgUAU a UAAUaUU
1298	UgAACUU a AGAAGcA	1507	AUAUAaU a UUCUaaU
1310	gcAAAGU a aAuACcA	1509	AUAUAUU U CUaAuAA
1310	GCAAAgU a aAUAcca	1509	aUaaUaU U CUAAUAA
1310	GcaAAgU a AAUAcca	1510	UAAUAUU C UaAuAAA
1350	AAAGCAU A AAUggU	1510	UAAUAUU C UaaUAAA
1358	AAAUUGU U ggGAugU	1510	UAUAuAU c UaaUAAA
1370	UgUuaUU C AGgUAUC	1510	UaaUaUU C UAUAUAA
1375	UUCAGgU A UCAGggU	1512	aUaUUUU A AUAAAgC
1377	CAGgUAU C AGggUCA	1515	UUCUAUU A AAgCAGa
1383	UCAGggU C AcUGgAG		
1405	cccCAgU U UACUcCA		

Table 14: Human IL-5 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
86	UACACGUA AGAA GUUCCA ACCAGAGAAACACACGUGUGUGUACAUUACUUGUA	UGGAGCU GGC UACGUGUA
151	GAGUAGAA AGAA GUGCCA ACCAGAGAAACACACGUGUGUGUACAUUACUUGUA	UGGACAU GCU UUCUACUC
172	UGGCUAUC AGAA GAGUUC ACCAGAGAAACACACGUGUGUGUACAUUACUUGUA	GAGCUU GCU GAUAGCCA
203	UGUACAGG AGAA GGAUUC ACCAGAGAAACACACGUGUGUGUACAUUACUUGUA	GAUUUUU GUU CCUGUACA

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Table 15: Mouse IL-5 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
75	AGCUAGGA AGAA GACAC ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GAC UCUAGCU
83	CCACACAC AGAA GAGAGU ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	ACUUCU GCU GUUUUUG
147	GAGCGCAC AGAA GUUUA ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	UGACAC GCU GUUUUUG
150	GUUAGUG AGAA GUCUG ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	CACACU GUC GUUUUUG
154	GUUUUUG AGAA GACAGC ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG
168	UGUUUUC AGAA GAGUUC ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GAGUUC GCU GUUUUUG
199	UGAGUGG AGAA GAGAGC ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GAGUUC GCU GUUUUUG
274	CCCCACG AGAA GUUGA ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GUC GUUUUUG
381	AUUCACG AGAA GUUUG ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	UGAAUU GUC GUUUUUG
454	CACCAUG AGAA GUUAG ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG
499	GUUUUUG AGAA GUUAC ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG
548	UUAUUGA AGAA GCAUUU ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG
701	GCAGGAG AGAA GAAUUU ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG
710	GAGAGGA AGAA GAGGGA ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG
870	AGUCAA AGAA GUUUG ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG
919	GUUUUUG AGAA GAGUUA ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG
1030	UAGAUAG AGAA GAGAGC ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG
1170	AUGGACA AGAA GUUUA ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG
1205	CAGAUUC AGAA GUUUA ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG
1402	GUUUUUG AGAA GUUUU ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG
1421	AGCAUAC AGAA GUUUU ACCAGGAAACACACGUGUGGUCACUAUACUUGUA	GUUUUU GCU GUUUUUG

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Table 16 : Mouse IL-5 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
75	AGCUGAGA AGAA GAACAC ACCAGGAAACACACGUGUGUACUUAUACUUGUA	GUUUUU GAC UUUACGU
85	CCAGACAC AGAA GAGGCU ACCAGGAAACACACGUGUGUACUUAUACUUGUA	ACUUCA GCU GUGUUGG
147	GAGCGCAC AGAA GUGUCA ACCAGGAAACACACGUGUGUACUUAUACUUGUA	UACACA GCU GUGUUGG
150	GGUAGCG AGAA GCUUG ACCAGGAAACACACGUGUGUACUUAUACUUGUA	CACAGU GUC GGUUACG
154	GUUGGUG AGAA GACAGC ACCAGGAAACACACGUGUGUACUUAUACUUGUA	GUUUGG GCU CACGAGC
168	UUUUUGC AGAA GAGGUC ACCAGGAAACACACGUGUGUACUUAUACUUGUA	GAGUUU GUU GACAGCA
199	UAGUGGG AGAA GGAAGC ACCAGGAAACACACGUGUGUACUUAUACUUGUA	GUUUUU GUC CUUACUA
274	UUUUUAG AGAA GUUUA ACCAGGAAACACACGUGUGUACUUAUACUUGUA	UCAAACU GUC GUUUGGG
381	AAUCCAG AGAA GCUUG ACCAGGAAACACACGUGUGUACUUAUACUUGUA	CGAGGA GUU CUUAGUU
454	CACUUGG AGAA GCUUAG ACCAGGAAACACACGUGUGUACUUAUACUUGUA	CUAGCU GCU CUUAGUU
499	GUUUUCC AGAA GUUAG ACCAGGAAACACACGUGUGUACUUAUACUUGUA	GUUACA GAU GCMAAAC
548	UAAUUGA AGAA GCNUU ACCAGGAAACACACGUGUGUACUUAUACUUGUA	AUUGCU GUU UUUUUUA
701	CGAGGAG AGAA GAAUU ACCAGGAAACACACGUGUGUACUUAUACUUGUA	AAUUUU GAU CUUUUUG
710	GAGGAGA AGAA GGAGA ACCAGGAAACACACGUGUGUACUUAUACUUGUA	UUUUUU GGC UUUUUUG
870	AGUCCAA AGAA GCUUG ACCAGGAAACACACGUGUGUACUUAUACUUGUA	CGAGCU GNC UUUAGAU
919	CUUGUUC AGAA GGAUUA ACCAGGAAACACACGUGUGUACUUAUACUUGUA	UGGUUA GAU GGAUUGG
1030	UAGUUGG AGAA GGAAGC ACCAGGAAACACACGUGUGUACUUAUACUUGUA	GUUUUU GCU CUUUGUA
1170	UUUCCAA AGAA GAUUA ACCAGGAAACACACGUGUGUACUUAUACUUGUA	UAAUUA GNC UGUUUUU
1205	CMAUUC AGAA GCUUA ACCAGGAAACACACGUGUGUACUUAUACUUGUA	UGAGCA GCU GGUUUUG
1402	CUUGGUA AGAA GGGGA ACCAGGAAACACACGUGUGUACUUAUACUUGUA	UUUUUA GUU UACUUGG
1421	AACUAUC AGAA GUUUU ACCAGGAAACACACGUGUGUACUUAUACUUGUA	AAAUUA GAU GUUUGUU

Table 17

Mouse *ref A* HH Target sequence

nt. Position HH Target Sequence nt. Position HH Target Sequence

19	AAUGGCU a caCaGgA	467	cCAGGCU c cuguUCg
22	aGCUCcU a cGUGGUG	469	AaGCcAU u AGcCAGC
26	CcUCcaU u GcGgACa	473	UuUgAGU C AGauCag
93	GAuCUGU U uCCCCUC	481	AGCGaAU C CAGACCA
94	AuCUGUU u CCCCUCA	501	AACCCCU U uCAGGUU
100	UuCCCCU C AUcUuAc	502	ACCCCUU u CAcGUUC
103	CCCUCAU C UUUCCcA	508	UuCAcGU U CCUADAG
105	CUCADCU U uCCcuCA	509	uCAcGUU C CUADAGA
106	UCAUCUU u CCcuCAG	512	cGUUCCU A UAGAgGA
129	CAGGCUU C UGGgCCu	514	UUCCTAU A GAgGAGC
138	GGgCCuU A UGUGGAG	534	GGGGAU A uGAcuUG
148	UGGAGAU C AUcGAaC	556	UGGcCCU C UGUUCC
151	AGAUCAU c GAaCAGC	561	CUCUGCU U CCAGGUG
180	AUGCGaU U CCGCUAu	562	UCUGCUU C CAGGUGA
181	UGCGaUU C CGCUAuA	585	aAgCCAU u AGcCAGc
186	UCCCGCU A uAAaUGC	598	GGCCCCU C CuCCUGa
204	GGGCGCU C aGCGGGC	613	CcCCUGU C CUcuCaC
217	GCAGaAU u CCuGGCG	616	CUGUCCU c uCaCAUC
239	CACAGAU A CCACCAA	617	gucCCUU C CUCAgCC
262	CCACCAU C AAGAUCA	620	CCUUCUU C AgCCaug
268	UCAAGAU C AAUGGCU	623	UCCUgcU u CCADCUc
276	AAUGGCU A CACAGGA	628	AUCCgAU u UUGAuA
301	UuCGaAU c UCCCUUG	630	CCgAUuU U UGAuAAc
303	CGaAUCU C CCUGGUC	631	CgAUuUU U GAuAAcC
310	CCCUGGU C ACCAAGG	638	UGgCCAU u GUGuCC
323	GGcCCCU C CUCcuga	661	CCGAGCU C AAGAUcU
326	uCCaCCU c ACCGGCC	667	UCAAGAU C UGCcGAG
335	CCGGCCU C AuCCaCA	687	CGgAAcU C UGGgAGC
349	AuGAaCU U GUgGGgA	700	GCUGCCU C GGUGGGG
352	AGaUcaU c GaAcAGc	715	AUGAGAU C UUCuUgC
375	GAUGGCU a CUAGGAG	717	GAGAUcU U CuUgCUG
376	AUGGucU C UccGgaG	718	AGAUCUU C uUgCUGU
378	GGCUaCU A UAGAGCU	721	UucUCCU c CauUGcG
391	CUGAcCU C UGCCCAG	751	AaGACAU U GAGGUGU
409	GCaGuAU C CAuAGcU	759	GAGGUGU A UUUACAG
416	CCgCAGU a UCCAuAg	761	GGUGUAU U UCAcGGG
417	CAuAGcU U CCAGAAC	762	GUGUAUU U CACGGGA
418	AuAGcUU C CAGAAC	763	UGUAUUU C ACCGGAC
433	UGGGgAU C CAGUGUG	792	CGAGGCU C CUUUUCu
795	GGCUCCU U UUCuCAA	1167	GAUGAGU U UuCCcCC
796	GCUCCUU U UCuCAAG	1168	AUGAGUU U uCCcCCA
797	CUCCUUU U CuCAAGC	1169	UGAGUUU u CCcCCAU
798	UCCUUUU C uCAAGCU	1182	AUGcUGU U aCCaUCA
829	UGGCCAU U GUGUCC	1183	UGcUGUU a CCaUCAg

834	AUUGUGU U CCGGACu	1184	GGccccU C CUcCUGa
835	UUGUGUU C CCGACuC	1187	GUccCuU c CUcaGCc
845	GACuCCU C CgUACGC	1188	UUaCCaU C aGGGCAG
849	CCUCCgU A CGCcGAC	1198	GGgAGuU u AGuCuGa
872	cCAGGCU C CUGUuCG	1209	CAGcCCU a caCCUUC
883	UuCGaGU C UCCAUGC	1215	cuGGCCU U aGCaCCG
885	CGaGUUU C CAUGCAG	1229	GGuCCCU u CCucAGc
905	GCGGCCU U CuGAuCG	1237	CCCAGcU C CUGCCCC
906	CGGCCUU C uGAuCGc	1250	CCAGcCU C CAGgCuC
919	GcGAGCU C AGUGAGC	1268	CCCAGCU C CuGCCcc
936	AUGGagU U CCAGUAC	1279	CCAUGGU c cCuuCcu
937	UGGagUU C CAGUACu	1281	gUGGgcU C AGCUgcG
942	UUCCAGU A CuUGCCA	1286	AUgAGuU u UccCCCA
953	GCucAU c CACAUGA	1309	CuCCUGU u CgAGUCu
962	AGAuGCU C GcCACCG	1315	cCCCAGU u CUAaCCC
965	CagUacU u gCCaGAc	1318	CAGUuCU A aCCCCgG
973	ACCGGAU U GAaGAGA	1331	gGGuCCU C CcCAGuC
986	GAgACcU u cAAGagu	1334	CuuUuCU C AaGCUGa
996	AGGACcU A UGAGACC	1389	ACGCGU C gGAAGCC
1005	GAGACCU U CAAGAGu	1413	CUGCAGU U UGADGcU
1006	AGACCUU C AAGAGuA	1414	UGCAGUU U GAUGcUG
1015	AGAGuAU C AUGAAGA	1437	GGGGCCU U GCUUGGC
1028	GAAGAGU C CUUCCaA	1441	CCUUGCU U GGCAACA
1031	GAGUCCU U UCAauGG	1467	GgaGUGU U CACAGAC
1032	AGUCCUU U CAauGGA	1468	gaGUGUU C ACAGACC
1033	GUCCUUU C AauGGAC	1482	CUGGCAU C uGUgGAC
1058	CCGGCCU C CAaCCCG	1486	CuUCgGU a GggAACU
1064	UaCACCU u GAuCCAa	1494	GACAACU C aGAGUUU
1072	GgCGuAU U CGUGUGC	1500	UCaGAGU U UCAGCAG
1082	UGUGCCU a CCCGaAa	1501	CaGAGUU U CAGCAGC
1083	aaGCCUU C CCGaAGu	1502	aGAGUUU C AGCAGCU
1092	CGaAaCU C AaCUUCU	1525	gGuGCAU c CCUGUGu
1097	CUCAaCU U CUGUCCC	1566	AUGGAGU A CCCUGAa
1098	UCAaCUU C UGUCCCC	1577	UGAaGCU A UAACUCG
1102	CUUCUGU C OCCAAGC	1579	AaGCUAU A ACUCGCC
1125	CAGCCCU A caCCUUC	1583	UAUAACU C GCCUgGU
1127	GCCaUAU a gCcUUAC	1588	CUcUCCU A GaGAggG
1131	cAUCCCU c agCacCA	1622	CCCAGCU C CUGCcCC
1132	AcaCCUU c cCagCAU	1628	UCCUGCU u CggUaGG
1133	UCCaUcU c CagCuUC	1648	CGGGGCU u CCCAADG
1137	UUUAcuU u AgCgCgc	1660	cUGaCCU C ugccCAG
1140	cCagCAU C CCUcAGC	1663	cuCUgCU U cCAGGuG
1153	GCACCAU C AACUuUG	1664	uCUgCUU c CAGGuGA
1158	ADCAACU u UGADGAG	1665	CUCgcUU u cGGAGgU
1680	GAAGACU U CUCCUCC		
1681	AAGACUU C UCCUCCA		
1683	GACUUCU C CUCCADU		
1686	UUCUCCU C CAUUGCG		
1690	CCUCCAU U GCGGACA		

1704	AUGGACU	U	CUUUGCU
1705	UGGACUU	C	UCUGGCU
1707	GACUUCU	C	UGGUCU
1721	uuUGAGU	C	AGAUCCAG
1726	GUCAGAU	C	AGCUCCU
1731	AUCAGCU	C	CUAAGGU
1734	AGCUCCU	A	AGGUGCU
1754	CaGUGCU	C	CCaAGAG

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Table 18

Human *rel A* HH Target Sequences

nt. Position HH Target Sequence nt. Position HH Target Sequence

19	AAUGGCU C GUCUGUA	467	GCAGGCU A UCAGUCA
22	GGCUCGU C UGUAGUG	469	AGGCUAU C AGUCAGC
26	CGUCUGU A GUGCAGG	473	UAUCAGU C AGCGCAU
93	GAACUGU U CCCCUC	481	AGCGCAU C CAGACCA
94	AACUGUU C CCCCUC	501	AACCCCU U CCAAGUU
100	UCCCCCU C AUCUCC	502	ACCCCUU C CAGGUC
103	CCUCAU C UCCCCG	508	UCCAAGU U CCUAUAG
105	CUCAUU U CCGGCA	509	CCAAGUU C CUUAAGA
106	UCAUCU C CCGGAG	512	AGUUCU A UAGAAGA
129	CAGGCU C UGGCCC	514	UUCCUU A GAGAGC
138	GGCCCU A UGUGAG	534	GGGACU A CGACCU
148	UGGAGU C AUUGAGC	556	UGGGCU C UGCUCC
151	AGAUCAU U GAGCAGC	561	CUCUGU U CCAGGUG
180	AUGGCU U CCGCUAC	562	UCUGCU C CAGGUA
181	UGGCUU C CGCUACA	585	GACCCAU C AGGCAGG
186	UUCGCU A CAAGUC	598	GGCCCU C CGCCUG
204	GGGCGU C CGGGGC	613	CGCCGU C CUCCUC
217	GCAGAU C CCAGGCG	616	CUGUCU U CCUCAUC
239	CACAGU A CCACCA	617	UGUCCU C CUCAUC
262	CCACAU C AAGAUCA	620	CCUCCU C AUCCAU
268	UCAAGU C AADGGCU	623	UCCUAC C CCAUCU
276	AADGGCU A CACAGGA	628	AUCCAU C UUUGACA
301	UGGCAU C UCCUGG	630	CCCAUCU U UGACAAU
303	CGCAUCU C CCGGUC	631	CCAUCU U GACAAUC
310	CCGUGU C ACCAAGG	638	UGACAAU C GUGCCC
323	GGACCU C CUCACCG	661	CCGAGCU C AAGAUU
326	CCUCCU C ACGGCC	667	UCAAGU C UGCGAG
335	CGGCGU C ACCCCA	687	CGAAAU C UGGCAGC
349	ACGAGCU U GUAGGAA	700	GCUGCU C GUGGGG
352	AGCUUGU A GGAAAGG	715	AUGAGU C UCCUAC
375	GAUGGU U CUAGAG	717	CAGAUU U CCUACU
376	AUGGCU C UAUAGG	718	AGAUUU C CUACUG
378	GGCUUCU A UGAGGU	721	UCUCCU A CUGUGU
391	CUGAGCU C UGCGCG	751	AGGACU U GAGGUG
409	GCUGAU C CACAGU	759	GAGGUGU A UUUCACG
416	CCACAGU U UCCAGAA	761	GGUGUAU U UACGGG
417	CACAGU U CCAGAAC	762	GUGUAU U CACGGGA
418	ACAGUU C CAGAAC	763	UGUAUU C ACGGGAC
433	UGGAUU C CAGUGU	792	CGAGGU C CUUUUG
795	GGUCCU U UUGCAA	1167	GAUGAU U UCCACC
796	GUCCUU U UGCAAG	1168	AUGAGU U CCCACCA
797	CUCCUU U CGCAAGC	1169	UGAGUU C CCACCAU
798	UCCUUU C GCAAGCU	1182	AUGGUGU U UCCUUCU
829	UGGCAU U GUGUCC	1183	UGGUGU U CCUUCU
834	AUUGGU U CCGACC	1184	GGUGUU C CUUCUG

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835	UUGUGUU C CGGACCC	1187	GUUUCUU U CUGGGCA
845	GACCCUU C CUAAGC	1188	UUUCCUU C UGGGCAG
849	CCUCCUU A CGCAGAC	1198	GGCAGAU C AGCCAGG
872	GCAGGCU C CUGUGCG	1209	CAGGCUU C GGCCUUG
883	UGCGUGU C UCCAUCC	1215	UCCGCUU U GGCCCCG
885	CGUGUCU C CAUGCAG	1229	GGCCCCU C CCAAGU
905	GCGGCUU U CCGACCG	1237	CCCAAGU C CUGCCCC
906	CGGCUUU C CGACCGG	1250	CCAGGCU C CAGCCCC
919	GGGAGCU C AGUGAGC	1268	CCUUGCU C CAGCCAU
936	AUGGAUU U CCAGUAC	1279	CCAUGGU A UCAGCUC
937	UGGAUUU C CAGUACC	1281	AUGGUUU C AGCUCUG
942	UUCCAGU A CCUGCCA	1286	AUCAGCU C UGGCCCA
953	GCCAGAU A CAGACGA	1309	CCCCUGU C CCAGUCC
962	AGACGCU C GUCACCG	1315	UCCAGU C CUAGCCC
965	CGAUUGU C ACCGGAU	1318	CAGUCCU A GCCCCAG
973	ACCGGAU U GAGGAGA	1331	AGGCCUU C CUCAGGC
986	GAAACGU A AAAGGAC	1334	CCUCCUU C AGGCUGU
996	AGGACAU A UGAGACC	1389	ACGUGU C AGAGGCC
1005	GAGACCU U CAAGAGC	1413	CUGCAGU U UGAUGAU
1006	AGACCUU C AAGAGCA	1414	UGCAGUU U GAUGAUG
1015	AGAGCAU C AUGAAGA	1437	GGGGCUU U GCUUGGC
1028	GAAGAGU C CUUUCAG	1441	CCUUGCU U GGCAACA
1031	GAGUCCU U UCAGCGG	1467	GCUGUGU U CACAGAC
1032	AGUCCUU U CAGCGGA	1468	CUGUGUU C ACAGACC
1033	GUCCUUU C AGCGGAC	1482	CUGGCAU C CGUCGAC
1058	CCGGCCU C CACCUCG	1486	CAUCUGU C GACAACU
1064	UCCACCU C GACGCAU	1494	GAACAACU C CGAGUUU
1072	GACGCAU U GCUGUGC	1500	UCCGAGU U UCAGCAG
1082	UGUGCCU U CCGCAG	1501	CCGAGUU U CAGCAG
1083	GUGCCUU C CCGCAGC	1502	CGAGUUU C AGCAGCU
1092	CGCAGCU C AGCUUCU	1525	AGGGCAU A CCGUGG
1097	CUCAGCU U CUGUCCC	1566	AUGGAGU A CCGUGAG
1098	UCAGCUU C UGUCCCC	1577	UGAGGCU A UAACUCG
1102	CUUCUGU C CCAAGC	1579	AGGCUAU A ACUCGCC
1125	CAGCCCU A UCCCUUU	1583	UAUAACU C GCCUAGU
1127	GCCCUAU C CCUUUAC	1588	CUCGCCU A GUGACAG
1131	UAUCCCU U UAGUCA	1622	CCCAGCU C CUGCUCC
1132	AUCCCUU U ACGUCAU	1628	UCCUGCU C CACUGGG
1133	UCCCUUU A CGUCADC	1648	CGGGGCU C CCCAUG
1137	UUUACGU C AUCCCUU	1660	AUGGCCU C CUUUCAG
1140	ACGUCAU C CCGAGC	1663	GCCUCCU U UCAGGAG
1153	GCACCAU C AACUAUG	1664	CCUCCUU U CAGGAGA
1158	AUCAACU A UGAUGAG	1665	CUCCUUU C AGGAGAU
1680	GAAGACU U CUCCUCC		
1681	AAGACUU C UCCUCCA		
1683	GACUUCU C CUCCAUU		
1686	UUCUCCU C CAUUGCG		
1690	CCUCCAU U GCGGACA		
1704	AUGGACU U CUCAGCC		

1705	UGGAGUU C UCAGCCC
1707	GACUUCU C AGCCCUG
1721	GCUGAGU C AGAUCAG
1726	GUCAGAU C AGCUCCU
1731	AUCAGCU C CUAAGGG
1734	AGCUCCU A AGGGGGU
1754	CUGCCCU C CCCAGAG

Table 19
 Mouse *rel A* HH Ribozyme Sequences
 nt. HH Ribozyme Sequence
 Sequence

19	UCCUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUU
22	CACCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCU
26	UGUCCGC	CUGAUGAGGCCGAAAGGCCGAA	ADGGAGG
93	GAGGGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGAUC
94	UGAGGGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGAU
100	GAAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGGGAA
103	AGGGAAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAGGG
105	UGAGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAG
106	CUGAGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGAUCA
129	AGGCCCA	CUGAUGAGGCCGAAAGGCCGAA	AAGCCUG
138	CUCCACA	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCC
148	GUUCGAU	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCA
151	GCUGUUC	CUGAUGAGGCCGAAAGGCCGAA	AUGAUUU
180	AUAGCGG	CUGAUGAGGCCGAAAGGCCGAA	AUCGCAU
181	UAUAGCG	CUGAUGAGGCCGAAAGGCCGAA	AADCGCA
186	GCAUUUA	CUGAUGAGGCCGAAAGGCCGAA	AGCGGAA
204	GCCCGCU	CUGAUGAGGCCGAAAGGCCGAA	AGCGGCC
217	CGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUACUGC
239	UUGGUGG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGUG
262	UGAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGG
268	AGCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGA
276	UCCUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUU
301	CCAGGGA	CUGAUGAGGCCGAAAGGCCGAA	AUUCGAA
303	GACCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCC
310	CCUUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
323	UCAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCC
326	GGCCGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGA
335	UGUGGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGG
349	UCCCCAC	CUGAUGAGGCCGAAAGGCCGAA	AGUUCAU
352	GCUGUUC	CUGAUGAGGCCGAAAGGCCGAA	AUGAUUU
375	CUCAUAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUC
376	CUCCGGA	CUGAUGAGGCCGAAAGGCCGAA	AGACCAU
378	AGCCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUAGCC
391	CUGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
409	AGCUAUG	CUGAUGAGGCCGAAAGGCCGAA	AUACUGC
416	CUAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUGCGG
417	GUUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCUAUG
418	GGUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGCUAU
433	CACACUG	CUGAUGAGGCCGAAAGGCCGAA	AUCCCCA
467	CGAACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCUGG
469	GCUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGCUU
473	CUGAUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUCAAA
481	UGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCGCU

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501	AACGUGA	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUU
502	GAACGUG	CUGAUGAGGCCGAAAGGCCGAA	AAGGGGU
508	CUAUAGG	CUGAUGAGGCCGAAAGGCCGAA	ACGUGAA
509	UCUAUAG	CUGAUGAGGCCGAAAGGCCGAA	AACGUGA
512	UCCUCUA	CUGAUGAGGCCGAAAGGCCGAA	AGGAACG
514	GCUCUC	CUGAUGAGGCCGAAAGGCCGAA	AUAGGAA
534	CAAGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCCC
556	GGAAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCGCA
561	CACCCUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
562	UCACCU	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGA
585	GCUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGCUU
598	UCAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCC
613	GUGAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACAGGGG
616	GADGUGA	CUGAUGAGGCCGAAAGGCCGAA	AGGACAG
617	GGCUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGGAC
620	CAUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGG
623	GAGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGA
628	UAUCAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGGAU
630	GUUAUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCCG
631	GGUUAUC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUCC
638	GGAACAC	CUGAUGAGGCCGAAAGGCCGAA	AUGGCCA
661	AGAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCG
667	CUCCGCA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGA
687	GCUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCCG
700	CCCCACC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGC
715	GCAAGAA	CUGAUGAGGCCGAAAGGCCGAA	AUCUCAU
717	CAGCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCU
718	ACAGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAUCUU
721	CGCAUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGAA
751	ACACCU	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUU
759	CGUGAAA	CUGAUGAGGCCGAAAGGCCGAA	ACACCU
761	CCCGUGA	CUGAUGAGGCCGAAAGGCCGAA	AUACACC
762	UCCCGUG	CUGAUGAGGCCGAAAGGCCGAA	AAUACAC
763	GUCCCGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
792	AGAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCUCG
795	UUGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCC
796	CUUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAGC
797	GCUUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAG
798	AGCUUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGGA
829	GGAACAC	CUGAUGAGGCCGAAAGGCCGAA	AUGGCCA
834	AGUCCCG	CUGAUGAGGCCGAAAGGCCGAA	ACACAAU
835	GAGUCCG	CUGAUGAGGCCGAAAGGCCGAA	AACACAA
845	GCGUACG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGUC
849	GUCGGCG	CUGAUGAGGCCGAAAGGCCGAA	ACGGAGG
872	CGAACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCUGG
883	GCAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCGAA
885	CUGCAUG	CUGAUGAGGCCGAAAGGCCGAA	AGACUCG
905	CGAUCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGC
906	GCGAUCA	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCG

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919	GCUCACU	CUGAUGAGGCCGAAAGGCCGAA	AGCUCGC
936	GUACUGG	CUGAUGAGGCCGAAAGGCCGAA	ACUCCAU
937	AGUACUG	CUGAUGAGGCCGAAAGGCCGAA	AACUCCA
942	UGGCAAG	CUGAUGAGGCCGAAAGGCCGAA	ACUGGAA
953	UCAUGUG	CUGAUGAGGCCGAAAGGCCGAA	ADGAGGC
962	CGGUGGC	CUGAUGAGGCCGAAAGGCCGAA	ADCAUCU
965	GUCUGGC	CUGAUGAGGCCGAAAGGCCGAA	AGUACUG
973	UCUCUUC	CUGAUGAGGCCGAAAGGCCGAA	ADCCGGU
986	ACUCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUC
996	GGUCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
1005	ACUCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUC
1006	UACUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCU
1015	UCUUCAU	CUGAUGAGGCCGAAAGGCCGAA	AUACUCU
1028	UUGAAAG	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUC
1031	CCAUUGA	CUGAUGAGGCCGAAAGGCCGAA	AGGACUC
1032	UCCAUUG	CUGAUGAGGCCGAAAGGCCGAA	AAGGACU
1033	GUCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAC
1058	CGGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGG
1064	UUGGADC	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUA
1072	GCACAGC	CUGAUGAGGCCGAAAGGCCGAA	AUACGCC
1082	UUUCGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCACA
1083	ACUUCGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUU
1092	AGAAGUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUG
1097	GGGACAG	CUGAUGAGGCCGAAAGGCCGAA	AGUUGAG
1098	GGGGACA	CUGAUGAGGCCGAAAGGCCGAA	AAGUUGA
1102	GCUUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAAG
1125	GAAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGGCUG
1127	GUAAGGC	CUGAUGAGGCCGAAAGGCCGAA	AUAUGGC
1131	UGGUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGGAUD
1132	AUGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUGU
1133	GAAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGA
1137	GCGCGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGUAAA
1140	GCUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGCUGG
1153	CRAAGUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGC
1158	CUCAUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGAU
1167	GGGGGAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCADC
1168	UGGGGGA	CUGAUGAGGCCGAAAGGCCGAA	AACUCAU
1169	AUGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAACUCA
1182	UGAUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAU
1183	CUGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGCA
1184	UCAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCC
1187	GGCUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGGAC
1188	CUGCCCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAA
1198	UCAGACT	CUGAUGAGGCCGAAAGGCCGAA	AACUCCC
1209	GAAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGGCUG
1215	CGGUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCAG
1229	GCUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGACC
1237	GGGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
1250	GAGCCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUGG

1268	GGGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
1279	AGGAAGG	CUGAUGAGGCCGAAAGGCCGAA	ACCAUGG
1281	CGCAGCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCAC
1286	UGGGGA	CUGAUGAGGCCGAAAGGCCGAA	AACUCAU
1309	AGACUCG	CUGAUGAGGCCGAAAGGCCGAA	ACAGGAG
1315	GGGUUAG	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGG
1318	CCGGGUU	CUGAUGAGGCCGAAAGGCCGAA	AGAACUG
1331	GACUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGACCC
1334	UCAGCUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAG
1389	GGCUUCC	CUGAUGAGGCCGAAAGGCCGAA	ACAGCGU
1413	AGCAUCA	CUGAUGAGGCCGAAAGGCCGAA	ACUGCAG
1414	CAGCAUC	CUGAUGAGGCCGAAAGGCCGAA	AACUGCA
1437	GCCAAGC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCC
1441	UGUUGCC	CUGAUGAGGCCGAAAGGCCGAA	AGCAAGG
1467	GUCUGUG	CUGAUGAGGCCGAAAGGCCGAA	ACACUCC
1468	GGUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AACACUC
1482	GUCCACA	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
1486	AGUUCOC	CUGAUGAGGCCGAAAGGCCGAA	ACCGAAG
1494	AAACUTU	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUC
1500	CUCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUGA
1501	GCGCUG	CUGAUGAGGCCGAAAGGCCGAA	AACUCUG
1502	AGCUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAACUTU
1525	ACACAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGCACC
1566	UUCAGGG	CUGAUGAGGCCGAAAGGCCGAA	ACUCCAU
1577	CGAGUUA	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCA
1579	GGCGAGU	CUGAUGAGGCCGAAAGGCCGAA	AUAGCUU
1583	ACCAGGC	CUGAUGAGGCCGAAAGGCCGAA	AGUUAUA
1588	CCUCUC	CUGAUGAGGCCGAAAGGCCGAA	AGGAGAG
1622	GGGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
1628	CUUACCG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGA
1648	CAUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGCCCOG
1660	CUGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
1663	CACCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
1664	UCACCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGA
1665	ACCUCCG	CUGAUGAGGCCGAAAGGCCGAA	AAGCGAG
1680	GGAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUUC
1681	UGGAGGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUU
1683	AADGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUC
1686	CGCAUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGAA
1690	UGUCCGC	CUGAUGAGGCCGAAAGGCCGAA	AUGGAGG
1704	AGCAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAU
1705	GAGCAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCA
1707	AAGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUC
1721	CUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	ACUCAAA
1726	AGGAGCU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAC
1731	ACCUUAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAU
1734	AGCACCU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCU
1754	CUCUUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCACUG

Table 20
Human *rel A* HH Ribozyme Sequences
nt. Position HH Ribozyme Sequences

19	UACAGAC	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUU
22	CACUACA	CUGAUGAGGCCGAAAGGCCGAA	ACGAGCC
26	CGUGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACAGACG
93	GAGGGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUUC
94	UGAGGGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGUU
100	GGAGAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGGGGA
103	CCGGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAGGG
105	UGCCGGG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAG
106	CUGCCGG	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGA
129	GGGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCUG
138	CUCCACA	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCC
148	GCUCAAU	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCA
151	GCUGCUC	CUGAUGAGGCCGAAAGGCCGAA	ADGAUCU
180	GUAGCGG	CUGAUGAGGCCGAAAGGCCGAA	AGCGCAU
181	UGUAGCG	CUGAUGAGGCCGAAAGGCCGAA	AAGCGCA
186	GCACUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCGGAA
204	GCCCGCG	CUGAUGAGGCCGAAAGGCCGAA	AGCGCCC
217	CGCCUGG	CUGAUGAGGCCGAAAGGCCGAA	AUGCUGC
239	UUGGUGG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGUG
262	UGAUCUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGG
268	AGCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGA
276	UCCUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUU
301	CCAGGGA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
303	GACCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCG
310	CCUUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
323	CGGUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGUCC
326	GGCCGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGG
335	UGGGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGG
349	UUCCUAC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCGU
352	CCUUUCC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGCU
375	CUCAUAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUU
376	CCUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAU
378	AGCCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCC
391	CCGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUCAG
409	AACUGUG	CUGAUGAGGCCGAAAGGCCGAA	AUGCAGC
416	UUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUGUGG
417	GUUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AACUGUG
418	GGUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAACUGU
433	CACACUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCCA
467	UGACUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCCUGC
469	GCUGACT	CUGAUGAGGCCGAAAGGCCGAA	AUAGCCU
473	AUGGCTU	CUGAUGAGGCCGAAAGGCCGAA	ACUGAUA
481	UGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AUGCGCU
501	AACUUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUU

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502	GAACUUG	CUGAUGAGGCCGAAAGGCCGAA	AAGGGGU
508	CUAUAGG	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGA
509	UCUAUAG	CUGAUGAGGCCGAAAGGCCGAA	AACUUGG
512	UCUUCUA	CUGAUGAGGCCGAAAGGCCGAA	AGGAACU
514	GCUCUUC	CUGAUGAGGCCGAAAGGCCGAA	AUAGGAA
534	CAGGUCG	CUGAUGAGGCCGAAAGGCCGAA	AGUCCCC
556	GGAAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCCGCA
561	CACCGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
562	UCACCTG	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGA
585	CCUGCCU	CUGAUGAGGCCGAAAGGCCGAA	ADGGGUC
598	GCAGGCG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCC
613	GAGGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCG
616	GAUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGACAG
617	GGAUAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGACA
620	AUGGGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGG
623	AAGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AUGAGGA
628	UGUCAA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGAU
630	ADUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGG
631	GAUUGUC	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGG
638	GGGGCAC	CUGAUGAGGCCGAAAGGCCGAA	AUUGUCA
661	AGAUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCG
667	CUCCGCA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGA
687	GUUGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCG
700	CCCCACC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGC
715	GUAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUCUCAU
717	CAGUAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCU
718	ACAGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAUUU
721	CACACAG	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGA
751	ACACCUC	CUGAUGAGGCCGAAAGGCCGAA	AUGUCCU
759	CGUGAAA	CUGAUGAGGCCGAAAGGCCGAA	ACACCUC
761	CCCGUGA	CUGAUGAGGCCGAAAGGCCGAA	AUACACC
762	UCCCGUG	CUGAUGAGGCCGAAAGGCCGAA	AAUACAC
763	GUCCCGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
792	CGAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCUCG
795	UUGCGAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCC
796	CUUGCGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAGC
797	GCUUGCG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAG
798	AGCUUGC	CUGAUGAGGCCGAAAGGCCGAA	AAAAGGA
829	GGAACAC	CUGAUGAGGCCGAAAGGCCGAA	AUGGCCA
834	GGUCCGG	CUGAUGAGGCCGAAAGGCCGAA	ACACAAU
835	GGGUCCG	CUGAUGAGGCCGAAAGGCCGAA	AACACAA
845	GCGUAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUC
849	GUCUGCG	CUGAUGAGGCCGAAAGGCCGAA	AGGGAGG
872	CGCACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCUCC
883	GCAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACACGCA
885	CUGCAUG	CUGAUGAGGCCGAAAGGCCGAA	AGACACG
905	CGGUCGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGC
906	CCGGUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCG
919	GCUCACU	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCC

936	GUACUGG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAU
937	GGUACUG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGCA
942	UGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	ACUGGAA
953	UCGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGC
962	CGGUGAC	CUGAUGAGGCCGAAAGGCCGAA	AUCGUCU
965	AUCCGGU	CUGAUGAGGCCGAAAGGCCGAA	ACGAUCG
973	UCUCCUC	CUGAUGAGGCCGAAAGGCCGAA	AUCCGGU
986	GUCCUUU	CUGAUGAGGCCGAAAGGCCGAA	ACGUUUC
996	GGUCUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCCU
1005	GCUCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
1006	UGCUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCU
1015	UCUUCAU	CUGAUGAGGCCGAAAGGCCGAA	AUGCUCU
1028	CUGAAAG	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUC
1031	CCGUCGA	CUGAUGAGGCCGAAAGGCCGAA	AGGACUC
1032	UCCGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGGACU
1033	GUCCGCU	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAC
1058	CGAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGG
1064	AUGCGUC	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGA
1072	GCAACGC	CUGAUGAGGCCGAAAGGCCGAA	AUGCGUC
1082	CUGCGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCACA
1083	GCUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCAC
1092	AGAAGCU	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCG
1097	GGGACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAG
1098	GGGACA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGA
1102	GCUGGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAAG
1125	AAAGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCGUG
1127	GUAAAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAGGGC
1131	UGACGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGAUA
1132	AUGACGU	CUGAUGAGGCCGAAAGGCCGAA	AAGGGAU
1133	GAUGACG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGGA
1137	CAGGGAU	CUGAUGAGGCCGAAAGGCCGAA	ACGUAAA
1140	GCUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGACGU
1153	CATAGUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGC
1158	CUCAUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGAU
1167	GGUGGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCAUC
1168	UGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AACTUCAU
1169	AUGGUGG	CUGAUGAGGCCGAAAGGCCGAA	AAACUCA
1182	AGAAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACACCAU
1183	CAGAAGG	CUGAUGAGGCCGAAAGGCCGAA	AAACCCA
1184	CCAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AAACACC
1187	UGCCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGAAAC
1188	CUGCCCA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAAA
1198	CCUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGCC
1209	CAAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUG
1215	CGGGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGA
1229	ACUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCC
1237	GGGGCAG	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGG
1250	GGGGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCUGG
1268	AUGGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGG

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1279	GAGCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCAUGG
1281	CAGAGCU	CUGAUGAGGCCGAAAGGCCGAA	AUAACAU
1286	UGGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAU
1309	GGACUGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGGGG
1315	GGGCUAG	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGA
1318	CUGGGGC	CUGAUGAGGCCGAAAGGCCGAA	AGGACTG
1331	GCCUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGCCU
1334	ACAGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGG
1389	GGCCUCU	CUGAUGAGGCCGAAAGGCCGAA	ACAGCGU
1413	AUCAUCA	CUGAUGAGGCCGAAAGGCCGAA	ACTGCAG
1414	CAUCAUC	CUGAUGAGGCCGAAAGGCCGAA	AACTUGCA
1437	GCCAAGC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCC
1441	UGUUGCC	CUGAUGAGGCCGAAAGGCCGAA	AGCAAGG
1467	GUCUGUG	CUGAUGAGGCCGAAAGGCCGAA	ACACAGC
1468	GGUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AACACAG
1482	GUCGACG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
1486	AGUUGUC	CUGAUGAGGCCGAAAGGCCGAA	ACGGAUG
1494	AAACUCG	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUC
1500	CUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCGGA
1501	GCUCUG	CUGAUGAGGCCGAAAGGCCGAA	AACUCGG
1502	AGCUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAACUCG
1525	CCACAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCCU
1566	CUCAGGG	CUGAUGAGGCCGAAAGGCCGAA	ACUCCAU
1577	CGAGUUA	CUGAUGAGGCCGAAAGGCCGAA	AGCCUCA
1579	GGCGAGU	CUGAUGAGGCCGAAAGGCCGAA	AUAGCCU
1583	ACUAGGC	CUGAUGAGGCCGAAAGGCCGAA	AGUUATA
1588	CUGUCAC	CUGAUGAGGCCGAAAGGCCGAA	AGGCGAG
1622	GGAGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
1628	CCAGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGA
1648	CAUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGCCCCG
1660	CUGAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCAU
1663	CUCUCGA	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGC
1664	UCUCCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGGAGG
1665	AUCUCCU	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAG
1680	GGAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUUC
1681	UGGAGGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUU
1683	AAUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUC
1686	CGCAAUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGAA
1690	UGUCCGC	CUGAUGAGGCCGAAAGGCCGAA	AUGGAGG
1704	GGCUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAU
1705	GGGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCA
1707	CAGGGCU	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUC
1721	CUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	ACUCAGC
1726	AGGAGCU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAC
1731	CCCUUAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAU
1734	ACCCCCU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCU
1754	CUCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGCAG

Table 21
Human rel A Hairpin Ribozyme/Target Sequences
nt. Position Hairpin Ribozyme sequence

		Substrate
90	UGAGGGG AGA GUUC ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	GAACU GUU CCCCCUCA
156	GCUGCUDG AGAA GCUC ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	GAGCA GCC CAGCAGC
362	GCCAUCCC AGAA GUCC ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	GAACU GCC GGAUGGC
413	GUUCUGGA AGAA GUGG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CCACA GUU UCCAGAAC
606	GAAGGACA AGAA GCAG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CUGCC GCC UGUCCUUC
652	UUGAGCUC AGAA GUGU ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	ACACU GCC GAGCUCAA
695	CCCACCGA AGAA GCUG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CAGCU GCC UCGGUGGG
853	AGGCTUGG AGAA GCGU ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	ACGCA GAC CCCAGCCU
900	GGUCGGAA AGAA GCCG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CGCGG GCC UUCGACC
955	UGACGAUC AGAA GUAU ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	AUACA GAC GAUCGUCA
1037	GUCGGUGG AGAA GCUG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CAGCG GAC CCACCGAC
1045	GGCCGGGG AGAA GUGG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CCACC GAC CCCCAGCC
1410	CAUCAUCA AGAA GCAG ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	CUGCA GUU UGAUGAUG
1453	ACAGCUGG AGAA GUGC ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	GCACA GAC CCAGCUGU
1471	GAUGCCAG AGAA GUGA ACCAGAGAAACACACGUGUGUGGUACAUAUACCUUGUA	UCACA GAC CUGGCNUC

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Table 22
Mouse *rel/A* Hairpin Ribozyme/Target Sequences
nt. Position Hairpin Ribozyme sequence

nt. Position	Hairpin Ribozyme sequence	Substrate
137	GUUGCTUC AGAA GUUC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GAACA GCC GAAGCAAC
273	GAGAUUCG AGAA GUUC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GAACA GUU CGAAUUCUC
343	GCCAUCCC AGAA GUUC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GGACU GCC GGGAUUGC
366	GGGACAGAG AGAA GCCU ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	AGGCU GAC CUCUGCCC
633	UUGAGCTUC AGAA GUGU ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	ACACU GCC GAGTUCNA
676	CCCAACCGA AGAA GCUC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GAGCU GCC UCGGUGGG
834	AGGCTUGG AGAA GCCU ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	ACGCC GAC CCCAGGCTU
881	GAUCAGAA AGAA GCCG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CGCGG GCC UUCUGAUC
1100	AGGUGUAG AGAA CGCG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CCGCA GCC CUACACCU
1205	GGGCAGAG AGAA GUGC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GCACC GUC CUCUGCCC
1361	GGGCTUCC AGAA CGGU ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	ACGCC GUC GGAAGCCC
1385	CAGCAUCA AGAA GCAG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CUGCA GUU UGAUGCTUG
1431	ACUCCUGG AGAA GUGC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GCACA GAC CCAGGAGU
1449	GAUGCCAG AGAA GUGA ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	UCACA GAC CUGGCAUC
1802	AAGUCCGG AGAA GCUG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CAGCU GCC CCCGACTU
2009	UGGCTUCCA AGAA GUCC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GGACA GAC UGGAGCCA
2124	UGGUGUCG AGAA GCAC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GUGCU GCC CGACACCA
2233	AUUCUGAA AGAA GCCA ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	UGGCC GCC UUCAGAAU
2354	UCAGUAAA AGAA GUCU ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	AGACA GCC UUUACUGA

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Table 23: Human TNF- α HH Ribozyme Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
28	GGCAGGU U CUCUCC	321	GUCAGAU C AUCUUCU
29	GCAGGUU C UCUUCCU	324	AGAUCAU C UUCUGA
31	AGGUUCU C UUCUCU	326	AUCAUCU U CUCGAAC
33	GUUCUCU U CUCUCA	327	UCAUCU C UCGAAC
34	UUCUCU C CUCUCAC	329	AUCUUCU C GAACCC
37	UCUUCU C UCAUA	352	AGCCUGU A GCCCAG
39	UUCUCU C ACAUACU	361	CCCAUGU U GUAGCA
44	CUCACAU A CUGACCC	364	ADGUUGU A GCAAAC
58	CACGGCU C CACUUC	374	AAACCU C AAGUGA
65	CCACCU C UCUCCC	391	GGCAGCU C CAGUGG
67	ACCCUCU C UCCUCG	421	AUGCCU C CUGGCA
69	CCUCUCU C CCUGGA	449	GAGAGAU A ACCAGCU
106	GCAUGAU C CGGAGC	468	GUGCAU C AGAGGC
136	AGGCGCU C CCAAGA	480	GGCCUGU A CCUACU
165	CAGGGCU C CAGGCG	484	UGUACCU C AUCUACU
177	CGGUGCU U GUUCUC	487	ACCUCAU C UACUCC
180	UGCUUGU U CCUCAGC	489	CUCAUCU A CUCCAG
181	GUUGGU C CUCAGCC	492	AUCUACU C CCAGUC
184	UGUUCU C AGCCUCU	499	CCAGGU C CUCUCA
190	UCAGCCU C UUCUCU	502	AGGUCCU C UUCAAG
192	AGCCUCU U CUCUUC	504	GUCCUCU U CAAGGC
193	GCUCUCU C UCUUCC	505	UCCUCU C AAGGCC
195	CUCUCU C CUUCUG	525	UGCCCU C CACCAU
198	UUCUCU U CCUGAU	538	AUGUGCU C CUAACC
199	UCUCCU C CUGAUC	541	UGCUCCU C ACCACA
205	UCCUGAU C GUGGAG	553	ACACCAU C AGCGCA
226	CCAGCU C UUCGCC	562	GCGCAU C GCGUCU
228	ACGUCU U CUGCUG	568	UCGCGU C UCUACC
229	CGCUCU C UGCUGC	570	GCGUCU C CUAACC
243	CUGCAU U UGAGUG	573	GUUCU A CCAACC
244	UGCACU U GGAGUA	586	CCAAGU C AACUCC
253	GAGUGAU C GGGCCC	592	UCAACU C CUCUCG
273	GAAGAGU C CCCCAG	595	ACCUCCU C UUGCCA
286	GGGACCU C UCCTAA	597	CUCCUCU C UGCAUC
288	GACCUU C UCUAUC	604	CUGCAU C AAGAGC
290	CCUCUCU C UAADCAG	657	CCUGGU A UGAGCC
292	UCUCUCU A AUCAGCC	667	AGCCAU C UAUUGG
295	CUCUAAU C AGCCUC	669	CCCAUCU A UUGGGA
302	CAGCCU C UGGCCA		

671	CADCUAU C UGGGAGG	960	UGGGAUU C AGGAAUG
682	GAGGGGU C UGCCAGC	1001	AACCACU A AGAAUUC
684	GGGGUCU U CCAGCUG	1007	UAAGAAU U CAAACUG
685	GGGUCUU C CAGCUGG	1008	AAGAAUU C AAACUGG
709	ACCGACU C AGCGCUG	1021	GGGGCCU C CAGAACU
721	CUGAGAU C AADGGGC	1029	CAGAACU C ACUGGGG
725	GADCAAU C GGCCCGA	1040	GGGGCCU A CAGCUUU
735	CCCGACU A UCUOGAC	1046	UACAGCU U UGADCCC
737	CGACUAA C UCGACUU	1047	ACAGCUU U GAUCCCU
739	ACTUACU C GACUUUG	1051	CUUUGAU C CCUGACA
744	CUCGACU U UGCGGAG	1060	CUGACAU C UGGAAUC
745	UCGACUU U GCGGAGU	1067	CUGGAUU C UGGAGAC
753	GCGGAGU C UGGGCAG	1085	GGAGCCU U UGGUUCU
763	GGCAGGU C UACUUUG	1086	GAGCCUU U GGUUCUG
765	CAGGUCU A CUUUGGG	1090	CUUUGGU U CUGGCCA
768	GUCUACU U UGGGAUC	1091	UUUGGUU C UGGCCAG
769	UCUACUU U GGGAUCA	1113	CAGGACU U GAGAGA
775	UUGGGAU C AUUGCCC	1124	AAGACCU C ACCUAGA
778	GGAUCAU U GCGGCUU	1129	CUCACTU A GAAAUUG
801	CGAACAU C CAACCUU	1135	UAGAAAU U GACACAA
808	CCAACTU U CCAAAAC	1151	UGGACCU U AGGCCUU
809	CAACCUU C CCAAAAC	1152	GGACCUU A GGCUCUC
820	AACGCCU C CCGGCC	1158	UAGGCCU U CCUCUCU
833	CCCAAU C CCUUUAU	1159	AGGCCUU C CUCUCUC
837	AUCCCUU U UAUUACC	1162	CCUCCCU C UCUCACG
838	AUCCCUU U AUUACCC	1164	UCCCUU C UCACAGU
839	UCCCUU A UUAACCC	1166	CCUCUCU C CAGAUUU
841	CCUUUAU U ACCCCCU	1174	CAGAUUU U UCCAGAC
842	CUUUUAU A CCCCCUC	1175	AGAUGUU U CCAGACU
849	AACCCCU C CUUCAGA	1176	GAUGUUU C CAGACUU
852	CCUCCCU U CAGACAC	1183	CCAGACU U CCUGAG
853	CCUCCCU C AGACACC	1184	CAGACUU C CUUGAGA
863	ACACCCU C AACCCCU	1187	ACTUCCU U CAGACAC
869	UCAACCU C UUCUGGC	1208	CAGCCCU C CCAACGG
871	AACUCU U CUGGCUC	1224	GCCAGCU C CCUCUUA
872	ACCUCUU C UGGCUCA	1228	GCUCCCU C UAUUUUA
878	UCUGGCU C AAAAAGA	1230	UCCCUU A UUUUUGU
890	AGAGAAU U GGGGGCU	1232	CCUCUUA U UAUUUUU
898	GGGGGCU U AGGGGUG	1233	CUUUAUU U AUGUUUG
899	GGGGGCU A GGGGUGG	1234	UCUUAUU A UGUUUUG
904	UUAGGGU C GGAACCC	1238	UUUAUUU U UGCACUU
917	CCAAGCU U AGAACUU	1239	UUUAUUU U GCACUUG
918	CAAGCUU A GAACUUU	1245	UUGCACU U GUGAUUA
924	UAGAACU U UAAGCAA	1251	UUGUGAU U AUUUUAU
925	AGAACUU U AAGCAAC	1252	UGUGAUU A UUUUAUA
926	GAACUUU A AGCAACA	1254	UGAUUAU U UAUUAUU
945	CACCAUU U CGAAACC	1255	GAUUAUU U AUUAUUU
946	ACCACUU C GAAACCU	1256	AUUUAUU A UUAUUUA
959	CUGGGAU U CAGGAUU	1258	UAUUUAU U AUUUUAU

1259	AUUUAUU A UUUUUUU	1440	UGUUUUU U AAAAUUU
1261	UUUUUUU U UUUUUUU	1441	GUUUUUU A AAUUUUU
1262	UUUUUUU U AUUUUUU	1446	UUAAAAU A UUAUCUG
1263	AUUUUUU A UUUUUUA	1448	AAAAUUU U AUUUUUU
1265	UUUUUUU U UUUUUUU	1449	AAAUUUU A UUUUUUU
1266	AUUUUUU U AUUUUUU	1451	AUUUUUU C UGUUUUU
1267	UUUUUUU A UUUUUUA	1456	AUUUUUU U AAGUUUU
1269	UUUUUUU U AUUUUUU	1457	UUUUUUU A AGUUUUU
1270	AUUUUUU A UUUUUUU	1461	AUUUUUU U GUUUUUU
1272	UUUUUUU U UUUUUUU	1464	AAGUUUU C UAAUUUU
1273	UUUUUUU U AUUUUUU	1466	GUUUUUU A AUAUUUU
1274	AUUUUUU A UUUUUUU	1479	UGUUUUU U UGUUUUU
1276	UUUUUUU U UUUUUUU	1480	GUUUUUU U UGUUUUU
1277	AUUUUUU U AUUUUUU	1494	CUUUUUU C AUUUUUU
1278	UUUUUUU A UUUUUUU	1498	UUUUUUU C AUUUUUU
1280	UUUUUUU U UUUUUUU	1501	CUUUUUU U GUUUUUU
1281	AUUUUUU U AUUUUUU	1512	GAGUUUU C UGUUUUU
1282	UUUUUUU A AUUUUUU	1517	CUUUUUU C UGUUUUU
1294	UUUUUUU A UUUUUUU	1528	AGUUUUU U GUUUUUU
1296	AUUUUUU U UUUUUUU	1533	GUUUUUU C UGUUUUU
1297	AUUUUUU U AUUUUUU	1537	UUUUUUU A AUUUUUU
1298	UUUUUUU A UUUUUUU	1540	CUUUUUU C GUUUUUU
1300	UUUUUUU U UUUUUUU	1546	UUUUUUU A CUUUUUU
1301	AUUUUUU U GGUUUUU	1549	GUUUUUU A UUUUUUU
1315	CGUUUUU A UUUUUUU	1551	CUUUUUU U AUUUUUU
1317	GGUUUUU C UUUUUUU	1552	UUUUUUU C AUUUUUU
1334	CUUUUUU A GUUUUUU	1566	GAGUUUU A AAGUUUU
1345	GUUUUUU U GUUUUUU	1572	UAAUUUU U GUUUUUU
1350	CUUUUUU C AUUUUUU	1576	GUUUUUU U AGUUUUU
1359	GAGUUUU U UUUUUUU	1577	GUUUUUU A GGUUUUU
1360	AUUUUUU U UUUUUUU		
1361	CUUUUUU U UUUUUUU		
1362	AUUUUUU C UUUUUUU		
1386	GAUUUUU A GUUUUUU		
1393	AGUUUUU U UUUUUUU		
1394	GUUUUUU C UUUUUUU		
1401	CUUUUUU A UUUUUUU		
1414	GUUUUUU C UUUUUUU		
1422	GUUUUUU U UUUUUUU		
1423	GUUUUUU C UUUUUUU		
1425	GUUUUUU U UUUUUUU		
1426	CUUUUUU U UUUUUUU		
1427	CUUUUUU U UUUUUUU		
1431	UUUUUUU U UUUUUUU		
1432	UUUUUUU A UUUUUUU		
1436	AUUUUUU U UUUUUUU		
1437	UUUUUUU U UUUUUUU		
1438	UUUUUUU U UUUUUUU		

Table 24: Human TNF- α Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
28	GGAAGAG CUGAUGAGGCCGAAAGGCCGAA ACCUGCC
29	AGGAAGA CUGAUGAGGCCGAAAGGCCGAA AACCUCC
31	AGAGGAA CUGAUGAGGCCGAAAGGCCGAA AGAACCU
33	UGAGAGG CUGAUGAGGCCGAAAGGCCGAA AGAGAAC
34	GUGAGAG CUGAUGAGGCCGAAAGGCCGAA AAGAGAA
37	UAUGUGA CUGAUGAGGCCGAAAGGCCGAA AGGAAGA
39	AGU AUGU CUGAUGAGGCCGAAAGGCCGAA AGAGGAA
44	GGGUCAG CUGAUGAGGCCGAAAGGCCGAA AUGUGAG
58	GAGGGUG CUGAUGAGGCCGAAAGGCCGAA AGCCGUG
65	GGGGAGA CUGAUGAGGCCGAAAGGCCGAA AGGGUGG
67	CAGGGGA CUGAUGAGGCCGAAAGGCCGAA AGAGGGU
69	UCCAGGG CUGAUGAGGCCGAAAGGCCGAA AGAGAGG
106	CGUCCCG CUGAUGAGGCCGAAAGGCCGAA AUC AUGC
136	UCUUGGG CUGAUGAGGCCGAAAGGCCGAA AGCGCCU
165	CCGCCUG CUGAUGAGGCCGAAAGGCCGAA AGCCGUG
177	GAGGAAC CUGAUGAGGCCGAAAGGCCGAA AGCACCG
180	GCUGAGG CUGAUGAGGCCGAAAGGCCGAA ACAAGCA
181	GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AAC AAGC
184	AGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGAACA
190	AGGAGAA CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
192	GAAGGAG CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
193	GGAAGGA CUGAUGAGGCCGAAAGGCCGAA AAGAGGC
195	CAGGAAG CUGAUGAGGCCGAAAGGCCGAA AGAAGAG
198	GAUCAGG CUGAUGAGGCCGAAAGGCCGAA AGGAGAA
199	CGAUCAG CUGAUGAGGCCGAAAGGCCGAA AAGGAGA
205	CUGCCAC CUGAUGAGGCCGAAAGGCCGAA AUCAGGA
226	GGCAGAA CUGAUGAGGCCGAAAGGCCGAA AGCGGGG
228	CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGAGCGU
229	GCAGGCA CUGAUGAGGCCGAAAGGCCGAA AAGAGCG
243	CACUCCA CUGAUGAGGCCGAAAGGCCGAA AGUGCAG
244	UCACUCC CUGAUGAGGCCGAAAGGCCGAA AAGUGCA
253	GGGGGCC CUGAUGAGGCCGAAAGGCCGAA AUCACUC
273	CCUGGGG CUGAUGAGGCCGAAAGGCCGAA ACUCUUC
286	UUAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGUCCC
288	GAUUAGA CUGAUGAGGCCGAAAGGCCGAA AGAGGUC
290	CUGAUUA CUGAUGAGGCCGAAAGGCCGAA AGAGAGG
292	GGCUGAU CUGAUGAGGCCGAAAGGCCGAA AGAGAGA
295	GAGGGCU CUGAUGAGGCCGAAAGGCCGAA AUUAGAG
302	UGGGCCA CUGAUGAGGCCGAAAGGCCGAA AGGGCUG

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321	AGAAGAU	CUGADGAGGCCGAAAGGCCGAA	AUCUGAC
324	UCGAGAA	CUGADGAGGCCGAAAGGCCGAA	AUGAUCU
326	GUUCGAG	CUGADGAGGCCGAAAGGCCGAA	AGAUGAU
327	GGUUCGA	CUGADGAGGCCGAAAGGCCGAA	AAGAUGA
329	GGGUUC	CUGADGAGGCCGAAAGGCCGAA	AGAAGAU
352	CAUGGGC	CUGADGAGGCCGAAAGGCCGAA	ACAGGCU
361	UUGCUAC	CUGADGAGGCCGAAAGGCCGAA	ACAUGGG
364	GGUUGC	CUGADGAGGCCGAAAGGCCGAA	ACAACAU
374	UCAGCUU	CUGADGAGGCCGAAAGGCCGAA	AGGUUUU
391	GCCACUG	CUGADGAGGCCGAAAGGCCGAA	AGCUGCC
421	UGGCCAG	CUGADGAGGCCGAAAGGCCGAA	AGGGCAU
449	AGCUGGU	CUGADGAGGCCGAAAGGCCGAA	AUCUCUC
468	GCCUCU	CUGADGAGGCCGAAAGGCCGAA	AUGGCCAC
480	GAUGAGG	CUGADGAGGCCGAAAGGCCGAA	ACAGGCC
484	AGUAGAU	CUGADGAGGCCGAAAGGCCGAA	AGGUACA
487	GGAGUA	CUGADGAGGCCGAAAGGCCGAA	ADGAGGU
489	CUGGGAG	CUGADGAGGCCGAAAGGCCGAA	AGAUGAG
492	GACCUUG	CUGADGAGGCCGAAAGGCCGAA	AGUAGAU
499	UGAAGAG	CUGADGAGGCCGAAAGGCCGAA	ACCUGGG
502	CCUUGAA	CUGADGAGGCCGAAAGGCCGAA	AGGACCU
504	GCCCUUG	CUGADGAGGCCGAAAGGCCGAA	AGAGGAC
505	GGCCCUU	CUGADGAGGCCGAAAGGCCGAA	AAGAGGA
525	AUGGGUG	CUGADGAGGCCGAAAGGCCGAA	AGGGGCA
538	GGGUGAG	CUGADGAGGCCGAAAGGCCGAA	AGCACAU
541	UGUGGGU	CUGADGAGGCCGAAAGGCCGAA	AGGAGCA
553	UGCGGCU	CUGADGAGGCCGAAAGGCCGAA	AUGGUGU
562	AGACGGC	CUGADGAGGCCGAAAGGCCGAA	AUGCGGC
568	GGUAGGA	CUGADGAGGCCGAAAGGCCGAA	ACGGCGA
570	CUGGUAG	CUGADGAGGCCGAAAGGCCGAA	AGACGGC
573	GGUCUGG	CUGADGAGGCCGAAAGGCCGAA	AGGAGAC
586	GGAGGUU	CUGADGAGGCCGAAAGGCCGAA	ACCUUGG
592	CAGAGAG	CUGADGAGGCCGAAAGGCCGAA	AGGUUGA
595	UGGCAGA	CUGADGAGGCCGAAAGGCCGAA	AGGAGGU
597	GADGGCA	CUGADGAGGCCGAAAGGCCGAA	AGAGGAG
604	GGCUCUU	CUGADGAGGCCGAAAGGCCGAA	AUGGCAG
657	GGGCUCA	CUGADGAGGCCGAAAGGCCGAA	ACCAGGG
667	CCAGAU	CUGADGAGGCCGAAAGGCCGAA	AUGGGCU
669	UCCAGA	CUGADGAGGCCGAAAGGCCGAA	AGAUUGG
671	CCUCCCA	CUGADGAGGCCGAAAGGCCGAA	AUAGADG
682	GCUGGAA	CUGADGAGGCCGAAAGGCCGAA	ACCCUC
684	CAGCUGG	CUGADGAGGCCGAAAGGCCGAA	AGACCCC
685	CCAGCUG	CUGADGAGGCCGAAAGGCCGAA	AAGACCC
709	CAGCGCU	CUGADGAGGCCGAAAGGCCGAA	AGUCGGU
721	GCCGAUU	CUGADGAGGCCGAAAGGCCGAA	AUCUCAG
725	UUGGGOC	CUGADGAGGCCGAAAGGCCGAA	AUUGAUC
735	GUCCAGA	CUGADGAGGCCGAAAGGCCGAA	AGUCGGG
737	AAGUCGA	CUGADGAGGCCGAAAGGCCGAA	AUAGUUC
739	CAAAGUC	CUGADGAGGCCGAAAGGCCGAA	AGAUAGU
744	CUCGGCA	CUGADGAGGCCGAAAGGCCGAA	AGUCGAG

745	ACUCGGC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCGA
753	CUGCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCGGC
763	CAAAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACCUGCC
765	CCCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGACCUU
768	GAUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
769	UGAUCCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUAGA
775	GGGCAAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCCAA
778	ACAGGGC	CUGAUGAGGCCGAAAGGCCGAA	AUGAUCC
801	AAGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUUCG
808	GUUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGG
809	CGUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUUG
820	GGCAGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCGUU
833	AUAAAGG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGGG
837	GGUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGAUU
838	GGGUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAGGGAU
839	GGGGUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAGGGA
841	AGGGGGU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAGG
842	GAGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AUAAGAG
849	UCUGAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGGU
852	GUGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGG
853	GGUGUCU	CUGAUGAGGCCGAAAGGCCGAA	AAGGAGG
863	AGAGGUU	CUGAUGAGGCCGAAAGGCCGAA	AGGGUGU
869	GCCAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
871	GAGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUU
872	UGAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGGU
878	UCUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGA
890	AGCCCCC	CUGAUGAGGCCGAAAGGCCGAA	AUUCUCU
898	CGACCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCCCC
899	CCGACCC	CUGAUGAGGCCGAAAGGCCGAA	AAGCCCC
904	GGGUUCC	CUGAUGAGGCCGAAAGGCCGAA	ACCCUAA
917	AAGUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGCUUGG
918	AAAGUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUG
924	UUGCUUA	CUGAUGAGGCCGAAAGGCCGAA	AGUUCUA
925	GUUGCUU	CUGAUGAGGCCGAAAGGCCGAA	AAGUUCU
926	UGUUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAAGUUC
945	GGUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGUG
946	AGGUUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGGU
959	AUUCUUG	CUGAUGAGGCCGAAAGGCCGAA	AUCCAG
960	CAUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUCCCA
1001	GAUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGUGGUU
1007	CAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUUA
1008	CCAGUUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUU
1021	AGUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCC
1029	CCCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AGUUCUG
1040	AAAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCC
1046	GGGAUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGUA
1047	AGGGAUC	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGU
1051	UGUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUCAAG
1060	GAUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCAG

1067	GUCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUUCACG
1085	AGAACCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCUCC
1086	CAGAACC	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUC
1090	UGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAAAG
1091	CUGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AACCAA
1113	UCUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUG
1124	UCUAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUU
1129	CAAUUUC	CUGAUGAGGCCGAAAGGCCGAA	AGGUGAG
1135	UUGUGUC	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUA
1151	AAGGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCA
1152	GAAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCC
1158	AGAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUA
1159	GAGAGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCU
1162	CUGGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGG
1164	AUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGAA
1166	ACAUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAGG
1174	GUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCUG
1175	AGUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AACAUCU
1176	AAGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAACAUC
1183	CUCAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUGG
1184	UCUCAAG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUG
1187	GUGUCUC	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGU
1208	CCAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUG
1224	AUAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGC
1228	AUAAUAU	CUGAUGAGGCCGAAAGGCCGAA	AGGGAGC
1230	ACAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGA
1232	AAACAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGG
1233	CAACAUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAG
1234	GCAAACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGA
1238	AAGUGCA	CUGAUGAGGCCGAAAGGCCGAA	ACAUAUA
1239	CAAGUGC	CUGAUGAGGCCGAAAGGCCGAA	AACAUUA
1245	UAUUCAC	CUGAUGAGGCCGAAAGGCCGAA	AGUGCAA
1251	AADAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUCACAA
1252	UAUAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAUCACA
1254	AAUAUAU	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
1255	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUC
1256	UAAAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1258	AAUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAAUAU
1259	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1261	AUAAUAU	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
1262	AAUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1263	UAUAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1265	AAUAUAU	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
1266	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1267	UAAAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAA
1269	AAUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
1270	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1272	AUAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
1273	AAUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU

1274	AAAUA	CUAUGAGGCCGAAAGGCCGAA	AAAUAAU
1276	GUAAUA	CUAUGAGGCCGAAAGGCCGAA	AUAAUA
1277	UGUAAU	CUAUGAGGCCGAAAGGCCGAA	AAUAAU
1278	CUGUAA	CUAUGAGGCCGAAAGGCCGAA	AAAUAA
1280	AUCUGA	CUAUGAGGCCGAAAGGCCGAA	AUAAUA
1281	CAUCUG	CUAUGAGGCCGAAAGGCCGAA	AAUAAU
1282	UCAUCG	CUAUGAGGCCGAAAGGCCGAA	AAAUAA
1294	AAAUAA	CUAUGAGGCCGAAAGGCCGAA	ACAUCA
1296	CCAAUA	CUAUGAGGCCGAAAGGCCGAA	AUACAU
1297	CCAAAU	CUAUGAGGCCGAAAGGCCGAA	AAUACU
1298	UCCCAA	CUAUGAGGCCGAAAGGCCGAA	AAAUCA
1300	UCUCCA	CUAUGAGGCCGAAAGGCCGAA	AUAAUA
1301	GUCUCC	CUAUGAGGCCGAAAGGCCGAA	AAUAAU
1315	CCCAGG	CUAUGAGGCCGAAAGGCCGAA	ACCCCG
1317	CCCCAG	CUAUGAGGCCGAAAGGCCGAA	AUACCC
1334	CAGCUC	CUAUGAGGCCGAAAGGCCGAA	ACAUCC
1345	CUGAGC	CUAUGAGGCCGAAAGGCCGAA	AGGCAG
1350	CAUGUC	CUAUGAGGCCGAAAGGCCGAA	AGCCAAG
1359	CACGGA	CUAUGAGGCCGAAAGGCCGAA	ACAUCC
1360	UACGGA	CUAUGAGGCCGAAAGGCCGAA	AAACAU
1361	UUCACG	CUAUGAGGCCGAAAGGCCGAA	AAACAUG
1362	UUUCAC	CUAUGAGGCCGAAAGGCCGAA	AAACAU
1386	AACAGC	CUAUGAGGCCGAAAGGCCGAA	AUUGUC
1393	ACAUGG	CUAUGAGGCCGAAAGGCCGAA	ACAGCC
1394	UACAUG	CUAUGAGGCCGAAAGGCCGAA	AACAGC
1401	AGGGGC	CUAUGAGGCCGAAAGGCCGAA	ACAUCC
1414	AGGCAC	CUAUGAGGCCGAAAGGCCGAA	AGGCCAG
1422	UCAAAG	CUAUGAGGCCGAAAGGCCGAA	AGGCAC
1423	AUCAA	CUAUGAGGCCGAAAGGCCGAA	AAGGCAC
1425	UAUCAA	CUAUGAGGCCGAAAGGCCGAA	AGAAGGC
1426	AUAUCA	CUAUGAGGCCGAAAGGCCGAA	AAGAAGG
1427	CAUAUC	CUAUGAGGCCGAAAGGCCGAA	AAAGAAG
1431	AAAACU	CUAUGAGGCCGAAAGGCCGAA	AUCAA
1432	AAAAAC	CUAUGAGGCCGAAAGGCCGAA	AUCAA
1436	UUUAAA	CUAUGAGGCCGAAAGGCCGAA	ACAUAA
1437	UUUUA	CUAUGAGGCCGAAAGGCCGAA	AACAUAA
1438	AUUUUA	CUAUGAGGCCGAAAGGCCGAA	AAACAU
1439	UAUUUA	CUAUGAGGCCGAAAGGCCGAA	AAACAU
1440	AUAUUU	CUAUGAGGCCGAAAGGCCGAA	AAAAAC
1441	AAUAUU	CUAUGAGGCCGAAAGGCCGAA	AAAAAC
1446	CAGAU	CUAUGAGGCCGAAAGGCCGAA	AUUUAA
1448	AUCAGU	CUAUGAGGCCGAAAGGCCGAA	AUAUUU
1449	AAUCAG	CUAUGAGGCCGAAAGGCCGAA	AUAUUU
1451	UUAUCA	CUAUGAGGCCGAAAGGCCGAA	AUAUAU
1456	ACAACU	CUAUGAGGCCGAAAGGCCGAA	AUCAGU
1457	GACAAC	CUAUGAGGCCGAAAGGCCGAA	AUCAGU
1461	UUUAGC	CUAUGAGGCCGAAAGGCCGAA	ACUUAU
1464	UUGUUU	CUAUGAGGCCGAAAGGCCGAA	ACAACU
1466	CAUUGU	CUAUGAGGCCGAAAGGCCGAA	AGACAAC

1479	GUCACCA	CUGAUGAGGCCGAAAGGCCGAA	AUCAGCA
1480	GGUCACC	CUGAUGAGGCCGAAAGGCCGAA	AUUCAGC
1494	AUUGAGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGUUG
1498	CAGCAAU	CUGAUGAGGCCGAAAGGCCGAA	AGUGACA
1501	CCUCAGC	CUGAUGAGGCCGAAAGGCCGAA	AUGAGUG
1512	GGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGGOCUC
1517	CCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
1528	CAGACAC	CUGAUGAGGCCGAAAGGCCGAA	ACUCCCU
1533	GAUUACA	CUGAUGAGGCCGAAAGGCCGAA	ACACAAC
1537	GGCCGAU	CUGAUGAGGCCGAAAGGCCGAA	ACAGACA
1540	GUAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AUUCAG
1546	UGAUAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGA
1549	CACUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUAGGC
1551	GCCACUG	CUGAUGAGGCCGAAAGGCCGAA	AUAGUAG
1552	CGCCACU	CUGAUGAGGCCGAAAGGCCGAA	AADAGUA
1566	CAACCUU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUC
1572	CCUAAGC	CUGAUGAGGCCGAAAGGCCGAA	ACCUUUA
1576	CUUCCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCAACC
1577	UCUUUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGCAAC

Table 25: Mouse TNF-a HH Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
66	UgGAAAU a GcuCCa	324	GgGUGAU C GGCCCC
101	GGCAGGU U CUGUcCC	347	GAGAgU u cCCAAaU
101	GGCAGGU u CUGUcCC	364	CCUCcCU C UCADUCAG
102	GCAGGU C UgUCCU	366	UCcCUU c ADUCAGU
102	gCAGGU c ugUCCU	366	UcCCU C auCAGU
106	GUUCUGU c CCUUCa	369	CUUCaU C AGuCUa
110	UgUCCU u UCACuA	376	CAGuCU a UGGCCA
111	gUCCU u CaCCAC	390	AgACCCU C AcaCUa
111	guCCU u CACuAc	396	ucaCacU C AGADCAU
112	UcCCU u ACuACU	401	cUCAGU C ADUCUCU
116	UuUCACU C AcUGgc	404	AGADCAU C UUCUCaA
137	GCCaCAU C uCCcUCc	406	ADCADCU U CUCAAAa
139	caCAuCU C CCUCcAg	406	AUCAUcU U cUCAAAA
177	GCADGAU C CGcGACG	407	UCAUCU C UCaaaau
207	AGGCaCU C CCCAAaA	409	AUCUCU C aAAauuC
228	GGGGCU C CAGAAU	409	AuCuCU c AaaaUUC
228	GGGGCU c CAGaacU	409	aUcUUCU c AAAauUC
236	CAGaaCU C CAGGCGG	432	AGCCUGU A GCCCAcG
236	CAGaACU c cAGgcGg		
249	GGUGCU a UgUCUcA	444	AcGUcGU A GCAAACC
249	GGUGCU a UGucUCA	501	AcGCCCU C CUGGCCA
261	UCAGCCU C UUCUCaU	560	gGgUUGU a CCUugUC
261	UCAgCCU C UUCUcau	560	GGguUGU A CCUugUC
263	AGCCUCU U CUCaUUC	564	UGUAACU u gUCUACU
263	AgCCUCU U CUcauUC	567	ACCUugU C UACUCCC
264	GCCUCU C UCauUCC	569	CUugUCU A CUCCCAG
264	gCCUCU C UcauUCC	572	gUCUACU C CCAGGUu
266	CUUCUCU C aUUCUG	572	GUCUaCU c CCAGguu
269	UUCUCaU U CCUGcUu	572	GuCUacU C CCAGGUu
270	UCUCaU C CUGcUuG	579	CCCAGGU u CUCUUA
276	UCCUGcU u GUGGCAG	580	CCAGguU c uCUUcAa
297	CCAGCU C UUCUGuC	580	CCAGguU c UCuUcaa
299	ACGUCU U CUGUCUa	582	AGGUUCU C UUCaagg
300	CGCUCU C UGUcUaC	582	AGGUuCU C UUCAAGG
304	CUuCUgU c uAcUGaa	584	GUuCUU U CAAGGGA
306	UcUGUcU a cUGAAcU	585	UuCUU C AAGGGAc
314	CUGaACU U cGgGUG	608	CcCGaCU a CgugCUC
315	UGaACU c GgGUGA	615	aCgUGcU C CUCaCCC
315	uGaaCU c GGGguGa	615	AcGUGCU C CUCACCC
324	gGGUGaU c GgUCCcC	618	UGCUU C ACCCACA

630	ACACCGU C AGCCGau	940	GuCUACU c cUCAGaG
630	ACACCGU C AgCCGaU	943	UACUccU C AGaGcCc
638	agcCgAU u uGCUaUc	972	UCUaaCU u AgAAAGg
643	aUUUGcU a uCUcAuA	972	ucUaaCU u AGAaAgG
645	UuGCuaU C UCauAAC	973	CUaACuU A GAAAggG
647	GCuaUCU C aUACCAG	984	AGgGgAU U auGGcuc
663	agAAaGU C AACCUCC	984	AGGGgaU U aUGgCUc
669	UCAACCU C CUCUCUG	985	GGGGauU a uGGcUCa
669	UcAAccU c cUCUCUG	997	UcAGaGU c CAACucU
672	ACCUCCU C UUCGCCg	1010	CuguGCU c AGAgCUU
674	CUCCUCU C UGCCgUC	1017	cAGAgCU U UcAaCAA
681	cUGCCgU C AagaGcC	1018	AGAgCUU U cAaCAAC
681	CUGCCgU C AAGAGCC	1019	GAgCUU c AaCAACu
681	CUGcCgU C aaGAgcC	1073	UgGGCCU c ucADgCA
734	CCCUUGU A UGAGCCC	1096	AAgGAcU C AAaugGG
734	CccUGGU a ugaGCCc	1106	aUGGcCU U uccGAAD
744	AGCCCAU a UAACUGG	1107	UGGcCUU u ccGAADu
746	CCCAUaU A cCUGGGA	1108	GGgCUU c cGzaUUC
759	GAgGAGU C uuCCAGc	1115	CcGAauU C ACUGGaG
759	GAGGAGU C UUCACAGC	1133	CGAAugU C CAuuCcU
761	GGaGUCU U CCAGCUG	1164	gagUGgU c AgGUUGc
762	GaGUCUU C CAGCUGG	1180	UcUgUcU c agaAUGA
786	ACCaACU C AGCGCUG	1203	aaGAuCU c AGGCCUU
798	CUGAgGU C AAUCuGC	1210	cAGGCCU U CCUaacU
802	GgUCAAU C uGCCCaA	1211	AGGCCUU C CUacCUu
812	CCCaAgU A cuUaGAC	1214	CCUCCCU a cCUuCAG
816	AgUAcuU a GACUUG	1218	CcuACcU u CaGACCu
821	uUaGACU U UGCGGAG	1218	CCuaCCU U CAGACCu
822	UaGACUU U GCgGAGU	1218	cCuACcU u cAgACCU
830	GCgGAGU C cGGGCAG	1218	CCUacCU u CAGAcCU
840	GGCAGGU C UACUUG	1219	CuaCCUU C AGACcuu
842	CAGGUCU A CUUUGGa	1219	CuAcCUU c agACcUU
842	CAGgucU a CUUugGA	1226	CagACCU U uCCAgAC
842	cagGuCU a CUUugGA	1226	CAGAcCU U UCCAGAC
845	GUUACU U UGGagUC	1227	agACCUU u CCAGACu
846	UCUACU U GGagUCA	1227	AGAcCUU U CCAGACU
852	UUGGagU C AUUGCuC	1228	GAcCUU C CAGACUc
855	GagUCAU U GCuCUU	1238	gACUcuU c CCUGAGG
887	AUCCaUU c ucUACCC	1262	CAGCCuU C CuCAcCa
891	AuuCuCU a CCCaGCC	1283	CCCCccU C uaUUUAU
905	CCcCaCU C UgaCCCC	1283	cCCCCCU C UAUUUAU
905	cCCCaCU c UgACCCC	1285	cCCCUU A UUUAUaU
905	CcCCACU c uGAcccC	1287	CcuCUAU u UauAuUU
914	GAcCCcU U uacUCUG	1287	CCUCUAU U UAUAUUU
915	ACCCCuU u acUCuGA	1288	CUUAUUU U AUaUUUG
919	CUUUAcU c ugaCCcC	1289	UCUAUUU A UaUUUGC
928	GACCCcU u UaUugUC	1293	UUUAUaU U UGCACUU
928	gAcCCCU U UAUUguC	1293	uUUaUaU u UGcAcUu
932	CCUUUAU U guCUaCU	1294	UUUAaUU U GCACUUA

1300	UUGCACU U aUuAUUu	1462	aCCuUGU u GCCuCCU
1303	CACuUaU u AuUuAUU	1470	GccuCcU C UUUUGcU
1304	acUuAUU A UUUADUA	1472	cuCcUCU U UUGcUUA
1306	UuAUUaU U UAUUAUU	1473	uCcUCUU U UGcUUaU
1307	uAUUAUU U AUUAUUU	1474	CcUCUUU U GcUUaUG
1307	UaUUAUU U AuUaUuU	1478	UUUUGcU U AUGUUa
1308	AUUAUUU A UUAUUUA	1479	UUUGcUU a UGuuuAa
1310	UauUaU U AUUUaUU	1479	UUUGcUU A UGUUUaa
1310	UAUUUAU U AUUUADU	1484	UUAUGUU U aaaAcAA
1310	UAUUUAU U AUUUADU	1498	AAAUauU U AUUAaAc
1311	AUUUAUU A UUUAUUU	1511	AcccAaU U GUUUaAA
1311	AUUUAUU A UUUAUUU	1514	cAaUUGU C UuAAuAA
1311	AuuUAUU A UuUauUU	1516	aUUGUCU U AAuAACG
1313	UUAUUaU U UAUUUaU	1529	CgcugAU u UGGUGAC
1313	UUAUUaU U UAUUUaU	1529	cGCUGAU U UGGUGAC
1313	uUAUUaU u UauUUaU	1530	gcUGAUU u gGUgacC
1314	UAUUUAU U AUUUADU	1530	GCUGAUU U GGUgACC
1314	UAUUUAU U AUUUADU	1563	UGaAcCU c UGcUCCC
1315	AUUUAUU A UUUADUA	1563	ugaaCCU C UGUCCCC
1317	UAUUUAU U UAUUAUU	1568	CUUGUCU C CCCAcGG
1318	AUUUAUU U AUUAUUU	1589	UGaCUGU A AUuGcCC
1319	UUUAUUU A UUAUUUA	1592	CUGUAAU u GcCCUAC
1326	AUUUAUU A UUUAUUU	1617	GAGAAAU A AAgAUcG
1328	UAUUUAU U UAUUUgC	1623	UAAAGaU c GCUUAaa
1329	AUUUAUU U AUUUgCu	1633	UUAaaaU a aaAAaCC
1330	UUUAUUU A UUUgCuU	25	AgGgaCU a gCCagGA
1332	UAUUUAU U UgCuUAU		
1333	AUUUAUU U gCuUAUG		
1337	auUUGCU U AuGAaUG		
1338	uUUGCUU A uGAaUGu		
1346	UGAADGU A UUUADUU		
1348	AAUGUAU U UAUUUGG		
1349	AUGUAUU U AUUUUGa		
1350	UGUAUUU A UUUUGaA		
1352	uAUuUAU u UGGaAGG		
1352	UAUUUAU U UGGaAGg		
1353	AUUUAUU U GGaAGgC		
1369	GGGUgU C CUUGaGG		
1398	gCUguCU U cAGACAg		
1398	GCUGuCU U cagaCAG		
1412	GACAUGU U UUCuGUG		
1413	ACADGUU U UCuGUGA		
1414	CADGUUU U CuGUGAA		
1415	AUGUUUU C uGUGAAA		
1415	AUGUUUU c UgugAaa		
1438	gaGCGU c CCCAccU		
1451	CUGGCTU C UcUaCCU		
1453	ggCCUCU C UaCCuUG		

Table 26: Mouse TNF- α Hammerhead Ribozyme Sequences

nt. Position	Mouse HH Ribozyme Sequence
25	UCCUGGC CUGAUGAGGCCGAAAGGCCGAA AGUCCCU
66	UGGGAGC CUGAUGAGGCCGAAAGGCCGAA AUUUGCA
101	GGGACAG CUGAUGAGGCCGAAAGGCCGAA ACCUGCC
101	GGGACAG CUGAUGAGGCCGAAAGGCCGAA ACCUGCC
102	AGGGACA CUGAUGAGGCCGAAAGGCCGAA AACUUGC
102	AGGGACA CUGAUGAGGCCGAAAGGCCGAA AACUUGC
106	UGAAAGG CUGAUGAGGCCGAAAGGCCGAA ACAGAAC
110	UGAGUGA CUGAUGAGGCCGAAAGGCCGAA AGGGACA
111	GUGAGUG CUGAUGAGGCCGAAAGGCCGAA AAGGGAC
111	GUGAGUG CUGAUGAGGCCGAAAGGCCGAA AAGGGAC
112	AGUGAGU CUGAUGAGGCCGAAAGGCCGAA AAAGGGA
115	GGCCAGU CUGAUGAGGCCGAAAGGCCGAA AGUGAAA
137	GGAGGGA CUGAUGAGGCCGAAAGGCCGAA AUGUGGC
139	CUGGAGG CUGAUGAGGCCGAAAGGCCGAA AGAUGUG
177	CGUCGCG CUGAUGAGGCCGAAAGGCCGAA AUCADGC
207	UUUGGGG CUGAUGAGGCCGAAAGGCCGAA AGUGCCU
228	AGUUCUG CUGAUGAGGCCGAAAGGCCGAA AAGCCCC
228	AGUUCUG CUGAUGAGGCCGAAAGGCCGAA AAGCCCC
236	CCGCCUG CUGAUGAGGCCGAAAGGCCGAA AGUUCUG
236	CCGCCUG CUGAUGAGGCCGAAAGGCCGAA AGUUCUG
249	UGAGACA CUGAUGAGGCCGAAAGGCCGAA AGGCACC
249	UGAGACA CUGAUGAGGCCGAAAGGCCGAA AGGCACC
261	AUGAGAA CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
261	AUGAGAA CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
263	GAAUGAG CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
263	GAAUGAG CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
264	GGAUGA CUGAUGAGGCCGAAAGGCCGAA AAGAGGC
264	GGAUGA CUGAUGAGGCCGAAAGGCCGAA AAGAGGC
266	CAGGAU CUGAUGAGGCCGAAAGGCCGAA AGAAGAG
269	AAGCAGG CUGAUGAGGCCGAAAGGCCGAA AUGAGAA
270	CAAGCAG CUGAUGAGGCCGAAAGGCCGAA AAUGAGA
276	CUGCCAC CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
297	GACAGAA CUGAUGAGGCCGAAAGGCCGAA AGCGUGG
299	UAGACAG CUGAUGAGGCCGAAAGGCCGAA AGAGCGU
300	GUAGACA CUGAUGAGGCCGAAAGGCCGAA AAGAGCG
304	UUCAGUA CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
306	AGUUCAG CUGAUGAGGCCGAAAGGCCGAA AGACAGA
314	CACCCCG CUGAUGAGGCCGAAAGGCCGAA AGUUCAG
315	UCACCCC CUGAUGAGGCCGAAAGGCCGAA AAGUACA

315	UCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AAGJUCA
324	GGGGACC	CUGAUGAGGCCGAAAGGCCGAA	AUCACCC
324	GGGGACC	CUGAUGAGGCCGAAAGGCCGAA	AUCACCC
347	AUUUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACUUCUC
364	CUGAUGA	CUGAUGAGGCCGAAAGGCCGAA	AGGGAGG
366	AACUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGA
366	AACUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGA
369	UAGAACU	CUGAUGAGGCCGAAAGGCCGAA	AUGAGAG
376	UGGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGAACUG
390	UGAGUGU	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCU
396	AUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUGA
401	AGAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUCOGAG
404	UUGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAUCU
406	UUUUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAU
406	UUUUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGADGAU
407	AUUUUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGA
409	GAADUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAU
409	GAADUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAU
409	GAADUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAU
432	CGUGGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCU
444	GGUUUGC	CUGAUGAGGCCGAAAGGCCGAA	ACGACGU
501	UGGOCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCU
560	GACAAGG	CUGAUGAGGCCGAAAGGCCGAA	ACAACCC
560	GACAAGG	CUGAUGAGGCCGAAAGGCCGAA	ACAACCC
564	AGUAGAC	CUGAUGAGGCCGAAAGGCCGAA	AGGUACA
567	GGGAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACAAGGU
569	CUGGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGACAAG
572	AACCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
572	AACCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
572	AACCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
579	UGAAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACCUGGG
580	UUGAAGA	CUGAUGAGGCCGAAAGGCCGAA	AACCUUG
580	UUGAAGA	CUGAUGAGGCCGAAAGGCCGAA	AACCUUG
582	CCUUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGAACCU
582	CCUUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGAACCU
584	UCCCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAC
585	GUCCCUU	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAA
608	GAGCACG	CUGAUGAGGCCGAAAGGCCGAA	AGUOGGG
615	GGGUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGCACGU
615	GGGUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGCACGU
618	UGUGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCA
630	AUCGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACGGUGU
630	AUCGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACGGUGU
638	GAUAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUCGGCU
643	UAUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGCAAUU
645	GGUAUGA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCAA
647	CUGGUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAUAGC

663	GGAGGUU	CUGAUGAGGCCGAAAGGCCGAA	ACUUCUU
669	CAGAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
669	CAGAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
672	CGGCAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGU
674	GAOGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGAG
681	GGCUCUU	CUGAUGAGGCCGAAAGGCCGAA	ACGGCAG
681	GGCUCUU	CUGAUGAGGCCGAAAGGCCGAA	ACGGCAG
681	GGCUCUU	CUGAUGAGGCCGAAAGGCCGAA	ACGGCAG
734	GGGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACCAAGG
734	GGGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACCAAGG
744	CCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCU
746	UCCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAUGGG
759	GCUGGAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCUC
759	GCUGGAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCUC
761	CAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGACUCC
762	CCAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGACUC
786	CAGCGCU	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGU
798	GCAGAUU	CUGAUGAGGCCGAAAGGCCGAA	ACUUCAG
802	UUGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUUGACC
812	GUCUAAG	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGG
816	CAAAGUC	CUGAUGAGGCCGAAAGGCCGAA	AAGUACU
821	CUCCGCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAA
822	ACUCCGC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUA
830	CUGCCCG	CUGAUGAGGCCGAAAGGCCGAA	ACUCCGC
840	CAAAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCGC
842	UCCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGACCCG
842	UCCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGACCCG
842	UCCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGACCCG
845	GACUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
846	UGACUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUAGA
852	GAGCAAU	CUGAUGAGGCCGAAAGGCCGAA	ACUCCAA
855	ACAGAGC	CUGAUGAGGCCGAAAGGCCGAA	AUGACTC
887	GGGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGAU
891	GGCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAU
905	GGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGGGG
905	GGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGGGG
905	GGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGGGG
914	CAGAGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUC
915	UCAGAGU	CUGAUGAGGCCGAAAGGCCGAA	AAGGGGU
919	GGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUAAAG
928	GACAAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUC
928	GACAAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUC
932	AGUAGAC	CUGAUGAGGCCGAAAGGCCGAA	AUAAGG
940	CUCUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
943	GGGCUCU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGUA
972	CCUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUGA
972	CCUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUGA
973	CCUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUGA
984	GAGCCAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCCCU

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984	GAGCCAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCCCU
985	UGAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AADCCCC
997	AGAGUUG	CUGAUGAGGCCGAAAGGCCGAA	ACUCUGA
1010	AAGCCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCACAG
1017	UUGUUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCUCUG
1018	GUUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AAGCUCU
1019	AGUUGUU	CUGAUGAGGCCGAAAGGCCGAA	AAAGCUC
1073	UGCAUGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCA
1096	CCCAUUU	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUU
1106	AUUCGGA	CUGAUGAGGCCGAAAGGCCGAA	AGCCCAU
1107	AAUUCGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCCA
1108	GAUUCG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCC
1115	CUCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AAUUCGG
1133	AGGAAUG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCG
1164	GCAACCU	CUGAUGAGGCCGAAAGGCCGAA	ACCAUCU
1180	UCAUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGACAGA
1203	AAGGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCU
1210	AGGUAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUG
1211	AAGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCU
1214	CUGAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGG
1218	AGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGG
1218	AGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGG
1218	AGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGG
1218	AGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGG
1219	AAGGUUCU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAG
1219	AAGGUUCU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAG
1226	GUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCG
1226	GUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCG
1227	AGUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUUCU
1227	AGUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUUCU
1228	GAGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGUC
1238	CCUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAGAGUC
1262	CUGUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCG
1283	AUAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGGGGG
1283	AUAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGGGGG
1285	AUAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGG
1287	AAAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGG
1287	AAAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGG
1288	CAAAUAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAG
1289	GCAAUUA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGA
1293	AAGUGCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAAA
1293	AAGUGCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAAA
1294	UAAGUGC	CUGAUGAGGCCGAAAGGCCGAA	AAAUUAA
1300	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AGUGCAA
1303	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAGUG
1304	UAUUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAAGU
1306	AAUAUAU	CUGAUGAGGCCGAAAGGCCGAA	AUAUAAA
1307	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUAU
1307	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUAU

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1308	UAAAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1310	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUU
1310	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUU
1310	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUU
1311	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1311	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1311	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1313	AUAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
1313	AUAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
1313	AUAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
1313	AUAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
1314	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1314	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1315	UUAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1317	AAUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
1318	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1319	UAAAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAA
1326	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1328	GCAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUU
1329	AGCAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1330	AAGCAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAA
1332	AUAAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUU
1333	CADUAGC	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1337	CADUCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAU
1338	ACAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AAGCAAA
1346	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUAUA
1348	CCAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
1349	UCCAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
1350	UCCCAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
1352	CCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUU
1352	CCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUU
1353	GCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1369	CCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	ACACCCC
1398	CUGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGACAGC
1398	CUGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGACAGC
1412	CACAGAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUC
1413	UCACAGA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGU
1414	UUCACAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAUG
1415	UUUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAACAU
1415	UUUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAACAU
1438	AGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUC
1451	AGGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCCAG
1453	CAAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGCC
1455	AACAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAGG
1462	AGGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGGU
1470	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGC
1472	UAAGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGAG
1473	AUAAGCA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGGA
1474	CAUAAGC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAGG
1478	UAAACAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAA

1479	UURAAACA	CUGAUGAGGCCCGAAAGGCCCGAA	AAGCAAA
1479	UURAAACA	CUGAUGAGGCCCGAAAGGCCCGAA	AAGCAAA
1484	UUGUUUU	CUGAUGAGGCCCGAAAGGCCCGAA	AACAUA
1498	GUUAGAU	CUGAUGAGGCCCGAAAGGCCCGAA	AADUUU
1511	UUAAGAC	CUGAUGAGGCCCGAAAGGCCCGAA	AUUGGU
1514	UUAUUA	CUGAUGAGGCCCGAAAGGCCCGAA	ACAAUUG
1516	CGUUUU	CUGAUGAGGCCCGAAAGGCCCGAA	AGACAU
1529	GUACCA	CUGAUGAGGCCCGAAAGGCCCGAA	AUCAGG
1529	GUACCA	CUGAUGAGGCCCGAAAGGCCCGAA	AUCAGG
1530	GGUCACC	CUGAUGAGGCCCGAAAGGCCCGAA	AADCAG
1530	GGUCACC	CUGAUGAGGCCCGAAAGGCCCGAA	AADCAG
1563	GGGAGCA	CUGAUGAGGCCCGAAAGGCCCGAA	AGGUUA
1563	GGGAGCA	CUGAUGAGGCCCGAAAGGCCCGAA	AGGUUA
1568	CGUGGG	CUGAUGAGGCCCGAAAGGCCCGAA	AGCAGG
1589	GGGCAU	CUGAUGAGGCCCGAAAGGCCCGAA	ACAGUA
1592	GUAGGG	CUGAUGAGGCCCGAAAGGCCCGAA	AUUACG
1617	CGAUUU	CUGAUGAGGCCCGAAAGGCCCGAA	AUUUCU
1623	UUUAAG	CUGAUGAGGCCCGAAAGGCCCGAA	AUCUUA
1633	GGUUUU	CUGAUGAGGCCCGAAAGGCCCGAA	AUUUUA

Table 27: Human 'TNF- α ' Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
46	AGCCGUGG AGAA GUUGGU ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	ACAUACU GAC CCACGGCU
54	GAGGGUGG AGAA GUUGGU ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	ACCCACG GCU CCACCCUC
185	GGAGAAAG AGAA GAGGAA ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	UUCCUCA GGC UCUUUCUC
201	CGCCACAG AGAA GGAAGG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CUUUCU GGU CGUGGCAG
230	GUCCAGCA AGAA GAAAGG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CUCUUCU GGC UGCUGGAC
234	CAAAGUGC AGAA GGCAGA ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	UCUGCCU GCU GCACUUUG
254	CTUCUGGG AGAA GAUCAC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GUGAUCG GGC CCCAGAGG
296	GGCCAGAG AGAA GAUUGG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CUAAUCA GGC CUCUGGCC
317	AGAAAGUG AGAA GACUCC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GCAGUCA GAU CAUCUUCU
387	GCCACUGG AGAA GCCCCU ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	AGGGGCA GCU CCAUGGGC
404	AUUGGCC AGAA GUUCAG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CUAAACC GGC GGGCCAAU
453	GCACCACC AGAA GGUUUG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	AUAAACA GCU GUGGGUGC
518	GGUGGAG AGAA GCUUUG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CAAGGCU GGC CCUCACAC
554	GGCAUCC AGAA GAUUGU ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	ACCAUCA GGC GCAUCGGC
565	UGGUAGGA AGAA GCGAUG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CAUCGCC GUC UCCUACCA
576	UGACCTUG AGAA GGUAGG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CCUACCA GAC CAAAGUCA
607	CCUUCUCC AGAA GGAAGA ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	UCUUCCA GCU GGAAGAGG
704	AGCGTUG AGAA GUCAAC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GGUGACC GAC UACAGCCU
726	GAUAGUCG AGAA GAUUGA ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	UCAAUCG GGC CGACUAUC
730	UCGAGUA AGAA GGCAGA ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	UGGGCCC GAC UAUUCUGA
824	GGGAUUG AGAA GGGGAG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CUCCCCU GGC CCAAUCCC
1042	GGGAUUA AGAA GUGGCC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GCCUACA GCU UUGAUCCC
1168	CUGGAAC AGAA GGAGAG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CUTUCCA GAU GUUUCGAG
1178	UCAAGGA AGAA GGAAGC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GUUUCCA GAC UUCUUUGA
1202	AUGGGAG AGAA GGGTUC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GAGCCCA GGC CUCCCCAU
1220	AUAGAGG AGAA GGTUCC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GGAGCCA GCU CCCUCUAU
1284	AUACAUUC AGAA GUAAAU ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	AUUUACA GAU GAUUGUAU
1340	UGAGCCAA AGAA GCUCCU ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	AGGAGCU GGC UUGGCCUA
1390	UACAUUGG AGAA GCCUUG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	AUAGGCU GUU CCCAUUGA

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1452	ACAACUUA	AGAA	GAUAAU	ACCAGAGAAACACACGUGUGUGUGUACAUUACCUUGUA	AUUAUUU	GAU	UAAGUUGU
1475	GUACACAA	AGAA	GCAUUG	ACCAGAGAAACACACGUGUGUGUGUACAUUACCUUGUA	CAUUGUU	GAU	UUGGUGAC
1513	CCUUGGGG	AGAA	GAGGCC	ACCAGAGAAACACACGUGUGUGUGUACAUUACCUUGUA	GGCUUUU	GGU	CCCCAGGG
1541	GAUAUGUA	AGAA	GAUUAU	ACCAGAGAAACACACGUGUGUGUGUACAUUACCUUGUA	GUAAUUG	GGC	UACUAUUC

Table 28: Mouse TNF- α Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
103	GUAAAAG AGAA GAACU ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	AGUUCU GUC CUUUUAC
256	UGAGAAGA AGAA GAGCA ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	UGUUA GGC UUUUUUA
272	CUCCACA AGAA GGAUG ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	CAUUCU GCU UUGGCGAG
301	GUUCAGUA AGAA GAGAG ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	CUUUCU GUC UACUGAAC
325	CUUUUGG AGAA GAUCAC ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	GUAGUG GUC CUCAAAGG
370	GGCCAUAG AGAA GAUAG ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	CUAUA GGU CUUUGGOC
383	GUUGAGG AGAA GGGCCA ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	UGGGCCA GAC CUCACACAC
397	AGAGAGUG AGAA GAGUG ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	ACAUA GGU CAUUCUUC
467	GGCCUCC AGAA GGUUU ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	AGAGCA GCU GAGUGGCC
546	AACCCUC AGAA GGCAC ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	GGUGCA GGC GAUGGGUU
549	UACAACC AGAA GCUUG ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	GGCAGC GGU GGGUUGUA
598	GUUGUUG AGAA GGUUG ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	CAAGCU GGC CCGACUAC
603	AGCAGUA AGAA GGGAG ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	CUGGCC GAC UACGUGCU
631	AGCAUUC AGAA GAGGU ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	ACCGUA GGC GAUUGCU
634	GAUGCAA AGAA GCUAC ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	GUCAGC GGU UUGCUUAC
675	CUUUGAC AGAA GAGGG ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	CUUUCU GGC GUCAAGAG
691	GUUUUGG AGAA GGGGU ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	AGUUUU GGC CCAAGGAC
764	CUUUUCC AGAA GGAUA ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	UUUUUA GCU GAGAGAGG
803	AGUACUUG AGAA GAUUA ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	UUAUU GGC CAGGUAU
895	AGAGUGG AGAA GGGUG ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	CUACCA GGC CCGAUUCU
906	GUAAAGG AGAA GAGUG ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	CAUUCU GAC CUCUUUAC
920	AUAAAGG AGAA GGUUA ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	UUAUU GAC CUCUUUAU
953	AGACACA AGAA GGGGC ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	GGUUUA GUC UGUGUUUC
1175	CNUUUGA AGAA GAGCA ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	UGUUUC GUC UACAGAUU
1220	CUUGAAG AGAA GAGGU ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	ACUUUA GAC CUUUUCCAG
1230	AGGAAGA AGAA GGAAG ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	CUUUUA GAC UUUUUUCU
1256	GUAGGGA AGAA GUUCU ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	AUGACA GGC UUUUUUAC
1274	UAGAGGG AGAA GGUUC ACCAGGAAACACACGUGUGUGUACAUUACUUGUA	AGACCA GGC CUCUUUAU

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Table 29: Human bcr/abl HH Target Sequence

Sequence ID No.	HH Target Sequence
<u>b2-a2</u> <u>Junction</u>	
20	UGACAUCA AUA AGGAGAGGC
21	GAGAGAGC CUU CAGGGGCAGU
22	AAGAGGC UUC AGGGGCAGUA

b3-a2
Junction

23	UAAGCAGAG UUC AAAAGGCCUUC
24	UCAAAGGC CUU CAGGGGCAGU
25	CAAAGGC UUC AGGGGCAGUA

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Table 30: Human *bcr-abl* HH Ribozyme Sequences

Sequence ID No.	HH Ribozyme Sequence
26	GGCUUCUCCU CUGAUGAGGCCGAAAGGCCGAA AUUGAUGGUA
27	ACUGGCCGCTG CUGAUGAGGCCGAAAGGCCGAA AGGGCUUCUUC
28	UACUGGCCGCU CUGAUGAGGCCGAAAGGCCGAA AAGGGCUUCU
29	GAAGGGCUUUU CUGAUGAGGCCGAAAGGCCGAA AACUCUGCUA
30	ACUGGCCGCTG CUGAUGAGGCCGAAAGGCCGAA AGGGCUUUUGA
31	UACUGGCCGCU CUGAUGAGGCCGAAAGGCCGAA AAGGGCUUUUG

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Table 31: RSV (1B) HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
10	GGCAAAU A AADCAAU	276	AAAAUAU A CUGAADA
14	AAUAAAU C AADUCAG	283	ACUGAAU A CAACACA
18	AAUCAAU U CAGCCAA	295	ACAAAUA A UGGCACU
19	ADCAADU C AGCCAAC	303	UGGCACU U UCCCUAU
54	CAADGAU A AUAACCC	304	GGCACUU U CCGUADG
57	UGADAAU A CACCACA	305	GCACUUU C CCGADGC
77	UGAUGAU C ACAGACA	309	UUUCCCU A UGCCAAU
94	AGACCGU U GUCACUU	317	UGCCAAU A UUCAUCA
97	CCGUUGU C ACUUGAG	319	CCAADAU U CAUCAAU
101	UGUCACU U GAGACCA	320	CAAUAAU C AUCAADC
110	AGACCAU A AUAACAU	323	UAUOCAU C AAUACAUG
113	CCADAUA A ACAUCAC	327	CAUCAU C AUGAUGG
118	AUAACAU C ACUAACC	337	GADGGGU U CUUAGAA
122	CADCAU A ACCAGAG	338	ADGGGUU C UUAAGAU
134	GAGACAU C AUAACAC	340	GGGUUCU U AGAUGC
137	ACAUCAU A ACACACA	341	GGUUCU A GAAUGCA
148	CACAAAU U UAUAUAC	350	AAUGCAU U GGCUAUA
149	ACAAAUU U AUUAUACU	356	UUGGCAU U AAGCCUA
150	CAAUUUU A UAUAUUU	357	UGGCAU A AGCCUAC
152	AAUUUAU A UACUUGA	363	UAAGCCU A CAAAGCA
154	UUUAUAU A CUUGADA	372	AAAGCAU A CUCCCAU
157	AUAUAU U GAUAAAU	375	GCAUAU C CCAUAUA
161	ACUUGAU A AAUCAUG	380	CUCCCAU A AUAUACA
165	GAUAAAU C AUGAUGG	383	CCAUAU A UACAAGU
176	AAUGCAU A GUGAGAA	385	AUAUAU A CAAGUAU
188	GAAAAU U GAUGAAA	391	UACAAGU A UGAUCUC
208	GCCACAU U UACAUUC	396	GUUGAU C UCAAUCC
209	CCACAUU U ACAUCCC	398	ADGAUCU C AAUOCAU
210	CACAUUU A CAUCCCU	402	UCUCAU C CAUAAAU
214	UUACAU U CUGGUC	406	AAUGCAU A AAUUUCA
215	UUACAU C CUGGUC	410	CAUAAAU U UCAACAC
221	UCCUGGU C AACUAUG	411	AUAUAU U CAACACA
226	GUCAAU A UGAAUUG	412	UAAAUUU C AACACAA
239	UGAAAU C UUACACA	421	ACACAAU A UUCACAC
241	AAACUAU U ACACAAA	423	ACAAUAU U CACACAA
242	AACUAU A CACAAAG	424	CAAUUAU C ACACAAU
251	ACAAAGU A GGAAGCA	432	ACACAAU C UAAAACA
261	AAGCACU A AAUAUA	434	ACAAUCU A AAACAAC
265	ACUAAAU A UAAAAAA	446	AACAACU C UAUGCAU
267	UAAUAU A AAAAAUA	448	CAACUCU A UGCAUAA
274	AAAAAAU A UACUGAA	454	UAUGCAU A ACUAUAC

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458 CAUAACU A UACUCCA
460 UAACUUAU A CUCCAUA
463 CUUAACU C CAUAGUC
467 ACUCCAU A GUCCAGA
470 CCAUAGU C CAGAUCC
489 UGAAAAU U AUAGUAA
490 GAAAAUU A UAGUAAU
492 AAAUUUAU A GUAAUUU
495 UUAUAGU A AUUUAUA

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NUC 37871

Table 32: RSV (1B) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
10	AUUGADU CUGAUGAGGCCGAAAGGCCGAA AUUUGCC
14	CGAADU CUGAUGAGGCCGAAAGGCCGAA AUUUAU
18	UUGGCG CUGAUGAGGCCGAAAGGCCGAA AUUGAU
19	GUUGGCU CUGAUGAGGCCGAAAGGCCGAA AAUUGAU
54	GGUGUAU CUGAUGAGGCCGAAAGGCCGAA AUCAUUG
57	UGUGGUG CUGAUGAGGCCGAAAGGCCGAA AUUAUCA
77	UGUCUGU CUGAUGAGGCCGAAAGGCCGAA AUCAUCA
94	AAGUGAC CUGAUGAGGCCGAAAGGCCGAA ACGGUCU
97	CUCAAGU CUGAUGAGGCCGAAAGGCCGAA ACACCGG
101	UGGUCUC CUGAUGAGGCCGAAAGGCCGAA AGUGACA
110	AUGUUAU CUGAUGAGGCCGAAAGGCCGAA ADGGUCU
113	GUGADGU CUGAUGAGGCCGAAAGGCCGAA AUUAUGG
118	GGUAGU CUGAUGAGGCCGAAAGGCCGAA ADGUUAU
122	CUUGGU CUGAUGAGGCCGAAAGGCCGAA AGUGAUG
134	GUGUUAU CUGAUGAGGCCGAAAGGCCGAA ADGUUC
137	UGUGUGU CUGAUGAGGCCGAAAGGCCGAA ADGAUGU
148	GUADAUA CUGAUGAGGCCGAAAGGCCGAA AUUUGUG
149	AGUAUAU CUGAUGAGGCCGAAAGGCCGAA AAUUGU
150	AAGUAUA CUGAUGAGGCCGAAAGGCCGAA AAUUG
152	UCAAGUA CUGAUGAGGCCGAAAGGCCGAA ADAAAUU
154	UAUCAAG CUGAUGAGGCCGAAAGGCCGAA ADUAUAA
157	AUUAUC CUGAUGAGGCCGAAAGGCCGAA AGUAUAU
161	CAUGAUU CUGAUGAGGCCGAAAGGCCGAA AUCAAGU
165	CAUUCAU CUGAUGAGGCCGAAAGGCCGAA AUUAUC
176	UUCUCAC CUGAUGAGGCCGAAAGGCCGAA ADGCAU
188	UUCAUC CUGAUGAGGCCGAAAGGCCGAA AGUUUC
208	GAAUGUA CUGAUGAGGCCGAAAGGCCGAA ADGUGGC
209	GGAAUGU CUGAUGAGGCCGAAAGGCCGAA AAUGUGG
210	AGGAADG CUGAUGAGGCCGAAAGGCCGAA AAADUGG
214	GACCAGG CUGAUGAGGCCGAAAGGCCGAA ADGUAAA
215	UGACCAG CUGAUGAGGCCGAAAGGCCGAA AAUGUAA
221	CAUAGUU CUGAUGAGGCCGAAAGGCCGAA ACCAGGA
226	CAUUUCA CUGAUGAGGCCGAAAGGCCGAA AGUUGAC
239	UGUGUAA CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
241	UUUGUGU CUGAUGAGGCCGAAAGGCCGAA ADAGUUU
242	CUUUGUG CUGAUGAGGCCGAAAGGCCGAA AAUAGUU
251	UGCUUCC CUGAUGAGGCCGAAAGGCCGAA ACUUUGU
261	UUUAUAU CUGAUGAGGCCGAAAGGCCGAA AGUGCUU
265	UUUUUA CUGAUGAGGCCGAAAGGCCGAA AUUUAGU
267	UAUUUUU CUGAUGAGGCCGAAAGGCCGAA ADUAUUA
274	UUCAGUA CUGAUGAGGCCGAAAGGCCGAA AUUUUUU
276	UAUUCAG CUGAUGAGGCCGAAAGGCCGAA ADUAUUU

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283	UGUGUUG	CUGAUGAGGCGAAAGGCCGAA	AUUCAGU
295	AGUGCCA	CUGAUGAGGCGAAAGGCCGAA	AUUUUGU
303	AUAGGGA	CUGAUGAGGCGAAAGGCCGAA	AGUGCCA
304	CAUAGGG	CUGAUGAGGCGAAAGGCCGAA	AAGUGCC
305	GCAUAGG	CUGAUGAGGCGAAAGGCCGAA	AAAGUGC
309	AUUGGCA	CUGAUGAGGCGAAAGGCCGAA	AGGGAUA
317	UGAUGAA	CUGAUGAGGCGAAAGGCCGAA	AUUGGCA
319	AUUGAUG	CUGAUGAGGCGAAAGGCCGAA	AUAUUGG
320	GAUUGAU	CUGAUGAGGCGAAAGGCCGAA	AUAUUGG
323	CAUGAUU	CUGAUGAGGCGAAAGGCCGAA	AUGAUAU
327	CCAUCAU	CUGAUGAGGCGAAAGGCCGAA	AUUGAUG
337	UUCUAAG	CUGAUGAGGCGAAAGGCCGAA	ACCCAUU
338	AUUCUAA	CUGAUGAGGCGAAAGGCCGAA	AAUCCAU
340	GCAUUCU	CUGAUGAGGCGAAAGGCCGAA	AGAACC
341	UGCAUUC	CUGAUGAGGCGAAAGGCCGAA	AAGAACC
350	UAUUGCC	CUGAUGAGGCGAAAGGCCGAA	AUGCAUU
356	UAGGCUU	CUGAUGAGGCGAAAGGCCGAA	AUGCCAA
357	GUAGGCU	CUGAUGAGGCGAAAGGCCGAA	AADGCCA
363	UGCUUUG	CUGAUGAGGCGAAAGGCCGAA	AGGCUUA
372	ADGGGAG	CUGAUGAGGCGAAAGGCCGAA	AUGCUUU
375	AUAUUGG	CUGAUGAGGCGAAAGGCCGAA	AGUAUUG
380	UGUAUAU	CUGAUGAGGCGAAAGGCCGAA	AUGGGAG
383	ACUUGUA	CUGAUGAGGCGAAAGGCCGAA	AUAUUGG
385	ADACUUG	CUGAUGAGGCGAAAGGCCGAA	AUAUAUU
391	GAGAUCA	CUGAUGAGGCGAAAGGCCGAA	ACUUGUA
396	GGAUUGA	CUGAUGAGGCGAAAGGCCGAA	AUCAUAC
398	ADGGAUU	CUGAUGAGGCGAAAGGCCGAA	AGAUCAU
402	AUUUAUG	CUGAUGAGGCGAAAGGCCGAA	AUUGAGA
406	UGAAAUU	CUGAUGAGGCGAAAGGCCGAA	AUGGAUU
410	GUGUUGA	CUGAUGAGGCGAAAGGCCGAA	AUUUAUG
411	UGUGUUG	CUGAUGAGGCGAAAGGCCGAA	AADUUUU
412	UUGUGUU	CUGAUGAGGCGAAAGGCCGAA	AAAUUUA
421	GUGUGAA	CUGAUGAGGCGAAAGGCCGAA	AUUGUGU
423	UUGUGUG	CUGAUGAGGCGAAAGGCCGAA	AUAUUGU
424	AUUGUGU	CUGAUGAGGCGAAAGGCCGAA	AAUAUUG
432	UGUUUUA	CUGAUGAGGCGAAAGGCCGAA	AUUGUGU
434	GUUGUUU	CUGAUGAGGCGAAAGGCCGAA	AGAUUGU
446	AUGCAUA	CUGAUGAGGCGAAAGGCCGAA	AGUUGUU
448	UUAUUCA	CUGAUGAGGCGAAAGGCCGAA	AGAGUUG
454	GUUAUGU	CUGAUGAGGCGAAAGGCCGAA	AUGCAUA
458	UGGAGUA	CUGAUGAGGCGAAAGGCCGAA	AGUAUUG
460	UAUGGAG	CUGAUGAGGCGAAAGGCCGAA	AUAUUGU
463	GACUAUG	CUGAUGAGGCGAAAGGCCGAA	AGUAUUG
467	UCUGGAC	CUGAUGAGGCGAAAGGCCGAA	AUGGAGU
470	CCAUCUG	CUGAUGAGGCGAAAGGCCGAA	ACUAUUG
489	UUACUAU	CUGAUGAGGCGAAAGGCCGAA	AUUUUAU
490	AUAUCUA	CUGAUGAGGCGAAAGGCCGAA	AAUUUUC
492	AAAUUAC	CUGAUGAGGCGAAAGGCCGAA	AUAUUUU
495	UUUAAAU	CUGAUGAGGCGAAAGGCCGAA	ACUAUUA

Table 33 : RSV (1C) HH target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
10	GGCAAU A AGAUAU	165	UACAUU A ACUAACG
16	UAAGAU U UGUAAG	169	UUUAACU A ACGCUU
17	AAGAAU U GAUAAGU	175	UAACGCU U UGGCUAA
21	AUUUGAU A AGUACCA	176	AACGCUU U GGUUAAG
25	GAUAAGU A CCACUA	181	UUUGGCU A AGGCAGU
31	UACCACU U AAAUUUA	192	CAGUGAU A CAUACAA
32	ACCACU A AAUUUA	196	GAUACAU A CAUCAA
36	CUAAAU U UAACUCC	201	AUACAAU C AAUUGA
37	UUAAAU U AACUCCC	206	AUCAAU U GAUUGC
38	UAAAUU A ACUCCU	216	AUGGCAU U GUGUUG
42	UUUAACU C CCUUGU	221	AUUGGUU U UGUGCAU
46	ACUCCU U GGUUAGA	222	UUGGUU U GUGCAUG
50	CCUUGU U AGAGUG	231	UGCAUGU U AUUACAA
51	CUUGGU A GAGUUG	232	GCAUGU A UUACAAG
67	CAGCAU U CAUUGAG	234	AUGUUAU U ACAAGUA
68	AGCAAU C AUUGAU	235	UGUUAU A CAUGUG
71	AAUUCAU U GAGUAG	241	UACAAGU A GUGAUU
76	AUUGAU A UGAUAA	247	UAGUUAU A UUUGCCC
81	GUAGAU A AAAGUA	249	GUGAUU U UGCCCCA
87	UAAAGU U AGAUUAC	250	UGAUUU U GCCCUAA
88	AAAGUU A GAUACA	256	UUGCCU A AUUAUA
92	GUAGAU U ACAAAU	259	CCCUAAU A AUAAUA
93	UUAGAU A CAAAUU	262	UAAUAU A AUUAUGU
100	ACAAAU U UGUUGA	265	UAAUAU A UUGUAGU
101	CAAAAU U GUUGAC	267	AUAUAU U GUAGUA
104	AAUUGU U UGACAU	270	AUAUGU A GUAAAU
105	AUUUGU U GACAUU	273	UUGUAGU A AAUCCA
120	AUGAAGU A GCAUUGU	278	GUAAAU C CAUUGC
125	GUAGCAU U GUUAAA	283	AUCCAU U UCACAC
128	GCAUUGU U AAAAUA	284	UCCAUU U CACAACA
129	CAUUGU A AAAUAU	285	CCAUUU C ACAACAA
135	UAAAAU A ACAUGCU	300	UGCCAGU A CUACAA
143	ACAUGCU A UACUGAU	303	CAGUACU A CAAAUU
145	AUGCUAU A CUGAUAA	316	UGGAGGU U AUUAUG
151	UACUGAU A AAUUAU	317	GGAGGUU A UAUAUGG
155	GAUAAU U AAUACAU	319	AGGUUAU A UAUGGA
156	AUAAAU A AUACAU	321	GUUAUAU A UGGGAA
159	AAUUAU A CAUUUA	338	AUGGAU U AACACAU
163	AAUACAU U UAACUA	339	UGGAUU A ACACAU
164	AUACAU U AACUAAC	346	AACACAU U GCUCUA

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350 CAUUGCU C UCAACCU
352 UUGCUCU C AACCUAA
358 UCAACCU A AUGGUCU
364 UAAUGGU C UACUAGA
366 AUGGUCU A CUAGAUG
369 GUCUACU A GAUGACA
379 UGACAAU U GUGAAAU
387 GUGAAAU U AAUUCU
388 UGAAAU A AAUUCU
392 AUUAAAU U CUCCAAA
393 UUAUAAU C UCCAAAA
395 AAUUCU C CAAAAA
405 AAAAAU A AGUGAUU
412 AAGUGAU U CAACAAU
413 AGUGAUU C AACAAUG
427 GACCAAU U AUUUGAA
428 ACCAAU A UAUGAU
430 CAUUUAU A UGAADCA
436 UAUGAU C AAUUAUC
440 AAUUAU U AUUUGAA
441 AUCAAU A UCUGAU
443 CAUUUAU C UGAUUUA
449 UCUGAU U ACUUGGA
450 CUGAAU A CUUGGAU
453 AAUUAU U GGAUUUG
458 CUUGGAU U UGAUUCU
459 UUGGAU U GAUCUA
463 AUUUGAU C UUAUCC
465 UUGAUCU U AAUCCAU
466 UGAUCCU A AUCCAU
469 UCUUAU C CAUAAU
473 AAUCCAU A AAUUAU
477 CAUAAU U AUUAUA
478 AUAAAU A UAAUUA
480 AAUUAU A AUUAUA
483 UUAUAU U AAUAUA
484 UUAUAU A AUUAUA
487 AAUUAU A UCAACUA
489 UUAUAU C AACUAGC
494 AUCAAU A GCAAUUC
501 AGCAAU C AAGUCA
507 UCRAUGU C ACUAACA
511 UGUCAU A ACACCAU
519 ACACCAU U AGUUAU
520 CACCAU A GUUAUA
523 CAUUAU U AAUAUA
524 AUUAU A AUUAUA

Table 34: RSV (1C) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
10	AAAUUCU CUGAUGAGGCCGAAAGGCCGAA AUUUGCC
16	CUUADCA CUGAUGAGGCCGAAAGGCCGAA AUUCUCA
17	ACUUUUC CUGAUGAGGCCGAAAGGCCGAA AAUUCUU
21	UGGUACU CUGAUGAGGCCGAAAGGCCGAA AUCAAAU
25	UAAUGGG CUGAUGAGGCCGAAAGGCCGAA ACUUUUC
31	UAAAUUU CUGAUGAGGCCGAAAGGCCGAA AGUGGUA
32	UUAAAUU CUGAUGAGGCCGAAAGGCCGAA AAGUGGU
36	GGAGUUA CUGAUGAGGCCGAAAGGCCGAA AUUUUAG
37	GGGAGUU CUGAUGAGGCCGAAAGGCCGAA AAUUUAA
38	AGGGAGU CUGAUGAGGCCGAAAGGCCGAA AAUUUUA
42	ACCAAGG CUGAUGAGGCCGAAAGGCCGAA AGUUAAA
46	UCUAAAC CUGAUGAGGCCGAAAGGCCGAA AGGGAGU
50	CACUUCU CUGAUGAGGCCGAAAGGCCGAA ACCAAGG
51	CCAUCUC CUGAUGAGGCCGAAAGGCCGAA AACCAAG
67	CUCAAUG CUGAUGAGGCCGAAAGGCCGAA AUUGGUG
68	ACUCAAU CUGAUGAGGCCGAAAGGCCGAA AAUGGCU
71	CAUACUC CUGAUGAGGCCGAAAGGCCGAA AUGAAUU
76	UUUADCA CUGAUGAGGCCGAAAGGCCGAA ACUCAAU
81	UAAUUUU CUGAUGAGGCCGAAAGGCCGAA AUCAUAC
87	GUAAUCU CUGAUGAGGCCGAAAGGCCGAA ACUUUUA
88	UGUAUUC CUGAUGAGGCCGAAAGGCCGAA AACUUUU
92	AUUUUUU CUGAUGAGGCCGAAAGGCCGAA AUCUAAU
93	AAUUUUG CUGAUGAGGCCGAAAGGCCGAA AADCUAA
100	UCAAACA CUGAUGAGGCCGAAAGGCCGAA AUUUUGU
101	GUCAAAC CUGAUGAGGCCGAAAGGCCGAA AAUUUUG
104	AUUGUCA CUGAUGAGGCCGAAAGGCCGAA ACUAAUU
105	CAUUGUC CUGAUGAGGCCGAAAGGCCGAA AACAAAU
120	ACAADGC CUGAUGAGGCCGAAAGGCCGAA ACUUCAU
125	UUUUUAC CUGAUGAGGCCGAAAGGCCGAA AUGCUAC
128	UAUUUUU CUGAUGAGGCCGAAAGGCCGAA ACAADGC
129	UUUUUUU CUGAUGAGGCCGAAAGGCCGAA AACAAUG
135	AGCAUGU CUGAUGAGGCCGAAAGGCCGAA AUUUUUA
143	AUCAGUA CUGAUGAGGCCGAAAGGCCGAA AGCAUGU
145	UUUUCAG CUGAUGAGGCCGAAAGGCCGAA AUAGCAU
151	AUUUAUU CUGAUGAGGCCGAAAGGCCGAA AUCAGUA
155	AUGUAUU CUGAUGAGGCCGAAAGGCCGAA AUUUUUC
156	AAUGUAU CUGAUGAGGCCGAAAGGCCGAA AAUUUUA
159	UUAAAUU CUGAUGAGGCCGAAAGGCCGAA AUUAUUU
163	UUAGUUA CUGAUGAGGCCGAAAGGCCGAA AUGUAUU
164	GUUAGUU CUGAUGAGGCCGAAAGGCCGAA AADGUUU
165	CGUUAGU CUGAUGAGGCCGAAAGGCCGAA AAUUGUA

169	AAAGCGU	CUGAUGAGGCCGAAAGGCCGAA	AGUUAUA
175	UUAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGCGUUA
176	CUUAGCC	CUGAUGAGGCCGAAAGGCCGAA	AAGCGUU
181	ACUGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCAAA
192	UUGUAUG	CUGAUGAGGCCGAAAGGCCGAA	ADCAUUG
196	UUGAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUAUC
201	UCAAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUGUAU
206	GCCAUUC	CUGAUGAGGCCGAAAGGCCGAA	AUUUGAU
216	CAAACAC	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAU
221	AUGCACA	CUGAUGAGGCCGAAAGGCCGAA	ACACAAU
222	CAUGCAC	CUGAUGAGGCCGAAAGGCCGAA	AACACAA
231	UUGUAAU	CUGAUGAGGCCGAAAGGCCGAA	ACAUGCA
232	CUUGUAA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGC
234	UACUUGU	CUGAUGAGGCCGAAAGGCCGAA	AUAACAU
235	CUACUUG	CUGAUGAGGCCGAAAGGCCGAA	AUAACA
241	AUAUCAC	CUGAUGAGGCCGAAAGGCCGAA	ACUUGUA
247	GGGCAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCACUA
249	UAGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUCAC
250	UAGGGC	CUGAUGAGGCCGAAAGGCCGAA	AUAUCA
256	UUAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AGGGCAA
259	AUAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAGG
262	ACAUAU	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
265	ACUACAA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUA
267	UUAUUA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAU
270	AUUUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUAU
273	UGGAUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUACAA
278	GAAAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUUUA
283	GUUGUGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGAU
284	UGUUGUG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGA
285	UUGUUGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGG
300	UUUGUAG	CUGAUGAGGCCGAAAGGCCGAA	ACUGGCA
303	CAUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGUACUG
316	CADUAU	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
317	CCAUUA	CUGAUGAGGCCGAAAGGCCGAA	AACUCC
319	UCCCAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAACCU
321	UUUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAAC
338	AUGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCCA
339	AADGUGU	CUGAUGAGGCCGAAAGGCCGAA	AADUCCA
346	UGAGAGC	CUGAUGAGGCCGAAAGGCCGAA	AUGUGUU
350	AGGUUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCAUUG
352	UUAGGUU	CUGAUGAGGCCGAAAGGCCGAA	AGAGCAA
358	AGACCAU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
364	UCUAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACCAUUA
366	CAUCUAG	CUGAUGAGGCCGAAAGGCCGAA	AGACCAU
369	UGUCAUC	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
379	AUUUCAC	CUGAUGAGGCCGAAAGGCCGAA	AUUGUCA
387	AGAAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCAC
388	GAGAAUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUUCA
392	UUUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUAU

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393	UUUUGGA	CUGAUGAGGCCGAAAGGCCGAA	AUUUUA
395	UUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAADUU
405	AADCACT	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUU
412	AUUGUUG	CUGAUGAGGCCGAAAGGCCGAA	ADCACTU
413	CAUUGUU	CUGAUGAGGCCGAAAGGCCGAA	AADCACT
427	UUCADAU	CUGAUGAGGCCGAAAGGCCGAA	ADUGGUC
428	ADUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AUUUGGU
430	UGAUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUUG
436	GAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	ADUCAUA
440	UCCAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUU
441	ADUCAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUU
443	UAUUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUUG
449	UCCAGU	CUGAUGAGGCCGAAAGGCCGAA	ADUCAGA
450	ADCCAAG	CUGAUGAGGCCGAAAGGCCGAA	AADUCAG
453	CAAUCC	CUGAUGAGGCCGAAAGGCCGAA	AGUAAUU
458	AAGAUCA	CUGAUGAGGCCGAAAGGCCGAA	ADCCAAG
459	UAAGAUC	CUGAUGAGGCCGAAAGGCCGAA	AAUCCAA
463	GGADUAA	CUGAUGAGGCCGAAAGGCCGAA	ADCAAUU
465	ADGGAUU	CUGAUGAGGCCGAAAGGCCGAA	AGAUCAA
466	UAUGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUCA
469	AUUUAUG	CUGAUGAGGCCGAAAGGCCGAA	AUUAAGA
473	UAUAADU	CUGAUGAGGCCGAAAGGCCGAA	ADGGAUU
477	UAADUUA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAUG
478	UUAAUUA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAUU
480	UAUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUUU
483	UGAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUAUAA
484	UUGADAU	CUGAUGAGGCCGAAAGGCCGAA	AADUADA
487	UAGUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUUAUUU
489	GCUAGUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUA
494	GAUUUGC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGAU
501	UGACAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUUGCU
507	UGUUAGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUGUA
511	ADGGUGU	CUGAUGAGGCCGAAAGGCCGAA	AGUGACA
519	AUUAACU	CUGAUGAGGCCGAAAGGCCGAA	ADGGUGU
520	UAUUUAC	CUGAUGAGGCCGAAAGGCCGAA	AADGGUG
523	UUADAUU	CUGAUGAGGCCGAAAGGCCGAA	ACTUADG
524	UUUADAU	CUGAUGAGGCCGAAAGGCCGAA	AACUAAU

Table 35: RSV (N) HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
9	GGCAAU A CAAAGU	217	GGUAGU U AUAGGG
21	GAUGGU C UAGCAA	218	GUAGUU A UAGGGA
23	UGGCUU U AGCAAAG	220	AUGUUU A UGGGAG
24	GGCUUU A GCAAAGU	229	GCGAGU C UAGGUA
32	GCAAAGU C AAGUUGA	231	GAUGUU A GGUAGG
37	GUCAAU U GAUGAU	235	UCUAGU U AGGAAG
45	GAUGAU A CACUCAA	236	CUAGGU A GGAAGG
50	AUACAU C AACAAAG	254	ACACCAU A AAAADAC
60	CAAAGU C AACUUCU	260	UAAAAU A CUCAGG
65	AUCAU U CUGUCAU	263	AAAUU C AGAGAG
66	UCAAU C UGUACU	277	GCGGUA A UCAUGU
70	CUUCU C AUCCAGC	279	GCGAUU C AUGUAA
73	CUGUCAU C CAGCAA	284	AUCAU A AAAGCA
82	AGCAAU A CACAUU	299	AUGGAGU A GAUGUA
89	ACACCAU C CAACGGA	305	UAGAGU A ACAACAC
108	AGGAGU A GUAGUA	315	AACAU C GUCAAG
111	AGAUAGU A UGUAU	318	ACAUU C AAGAUU
113	AUAGUAU U GAUACU	326	AGACAU U AUGGAA
117	UAUGAU A CUCCUAA	327	AGACAU A AUGGAA
120	UGAUACU C CUAAUA	346	AUGAAU U UGAAGG
123	UACUCCU A AUUAGU	347	UGAAAU U GAAGGU
126	UCCUAAU U AUGAGU	355	GAAGGU U AACAUU
127	CCUAAU A UGAAGG	356	AAGGUU A ACAUUG
146	AACACU C AAUAGU	361	UUAACU U GCGAGC
150	CAUCAU A AGUUAU	370	GCAAGU U AACACU
154	AAUAGU U AUGGGC	371	CAAGCU A ACAACU
155	AUAAGU A UGUGGA	383	CUGAAU U CAADCA
166	GGCAU U AUUAUC	384	UGAAAU C AAUCAA
167	GCAUGU A UUAUCA	389	UUCAAU C AACAUU
169	AUGUUAU U AAUCACA	395	UCAACU U GAGAGG
170	UGUUAU A AUCACAG	401	UUGAGU A GAUUAU
173	UAUUAU C ACAGAAG	406	AUAGAU C UAGAAA
186	AGAUUU A AUCAUA	408	AGAAU A GAAAUU
189	UGCUAU C AUAAAU	415	AGAAAU C CUACAA
192	UAUUAU A AAUUCAC	418	AAAUUU A CAAAAA
196	CAUAAU U CACUGG	431	AAAUUU A AAAGAA
197	AUAAAU C ACUGGU	449	GAGAGU A CUCCAG
205	ACUGGU U AAUAGU	453	GGUAGU C CAGAAU
206	CUGGUU A AUAGGU	460	CCAGAU A CAGGCU
209	GGUUAU A GGUAGU	472	CAUACU C UCCUAG
213	AAUAGU A UGUUAU	474	UGACUU C CUGAUU

480	UCCUGAU U GUGGGAU	696	UUUUGGU A UAGCACA
491	GG AUGAU A AUUAUUAU	698	UUGGUUAU A GCACAAU
494	UGAUAAU A UUAUGUA	706	GCACAAU C UUCUACC
496	AUAUAUAU U AUGUAUA	708	ACAADCU U CUACCAG
497	UAUAUAU A UGUUAUAG	709	CAADCUU C UACCAGA
501	AUUUAUGU A UAGCAGC	711	AUCUUCU A CCAGAGG
503	UAUGUAU A GCAGCAU	726	UGGAGU A GAGUUGA
511	GCAGCAU U AGUAUAUA	731	GUAGAGU U GAAGGGA
512	CAGCAU A GUUAUAUA	740	AAGGGAU U UUGCAG
515	CAUUAU A AUAAUA	741	AGGGAU U UUGCAGG
518	UAGUAU A ACTUAUU	742	GGGAUUU U UGCAGGA
522	AUAUAU A AAUUAAGC	743	GGAUUUU U GCAGGAU
526	ACTUAUU U AGCAGCA	751	GCAGGAU U GUUAUAG
527	CUAAAUU A GCAGCAG	754	GGAUUGU U UADGAU
544	GACAGAU C UGGUCUU	755	GADUGUU U AUGAAUG
549	AUCUGGU C UUAACAGC	756	AUUGUUU A UGUAUAGC
551	CUUGGUU U ACAGGCG	766	AAUGCCU A UGGUGCA
552	UGGUCUU A CAGGCGU	787	GUGAGU U ACGGUGG
563	CGUGAU U AGGAGAG	788	UGADGUU A CGGUGGG
564	CGUGAUU A GGAGAGC	800	GGGAGU C UUAAGCA
573	GAGAGU A AUUAUAGU	802	GGAGUUU U AGCAAAA
576	AGCUAAU A AUGUCCU	803	GAGUCUU A GCAAAAU
581	AUAUAGU C CUAAAAA	811	GCAAAAU C AGUUAUA
584	AUGUCCU A AAAAAUG	815	AAUCAGU U AAAAAUA
603	GAAACGU U ACAAAGG	816	AUCAGUU A AAAUAU
604	AAACGUU A CAAAGGC	822	UAAAAU A UUAUGUU
613	AAAGGUU U ACUAACC	824	AAAAUAU U AUGUAAG
614	AAGGUU A CUACCCA	825	AAUAUUU A UGUUAGG
617	GUUAU A CCAAGG	829	AUUAUUGU U AGGACAU
629	AGGACAU A GCCAACA	830	UUUAUGU A GGACAGG
640	AAACGUU U CUADGAA	840	ACADGUU A GUGUGCA
641	ACAGGUU C UAUUAAG	866	AACAAGU U GUUGAGG
643	AGCUUCU A UGAAGUG	869	AAGUUGU U GAGGUUU
652	GAAGUGU U UGAAAAA	875	UUGAGGU U UADGAU
653	AAGUGUU U GAAAAAC	876	UGAGGUU U AUGAAUA
663	AAAACAU C CCCACUU	877	GAGGUUU A UGRAUAU
670	CCCEACU U UAUAUAU	883	UAUGAAU A UGOCCAA
671	CCACUUU U AUAGAUG	895	CAAAAAU U GGGUGGU
672	CCACUUU A UAGADGU	913	GCAGGAU U CUACCAU
674	ACUUUAU A GAUGUUU	914	CAGGAUU C UACCAUA
680	UAGAUGU U UUGGUUC	916	GGAUUCU A CCAUAUA
681	AGAUGUU U UUGGUCA	921	CUACCAU A UAUUGAA
682	GAUGUUU U UGUUCAU	923	ACCAUAU A UUGAACA
683	AUGUUUU U GUUCAUU	925	CAUAUAU U GAACAAC
686	UUUUUGU U CAUUUUG	943	AAAGCAU C AUUAUAUA
687	UUUUGUU C AUUUUGG	946	GCAUCAU U AUUAUCU
690	UGUUCAU U UUGGUUAU	947	CAUCAUU A UUAUCUU
691	GUUCAUU U UGGUAUA	949	UCAUAUU U AUGUUUG
692	UUCAUUU U GGUUAUAG	950	CAUAUAU A UCUUUGA

952 UUAUUAU C UUUGACU
954 AUUAUCU U UGACUCA
955 UUAUCUU U GACUCAA
960 UUUGACU C AAUOUCC
964 ACUCAAU U UCCUCAC
965 CUCAAUU U CCUCACU
966 UCAAUUU C CUCACUU
969 AUUUCCU C ACUCCUC
973 CCUCACU U CUCCAGU
974 CUCACUU C UCCAGUG
976 CACUUCU C CAGUGUA
983 CCAGUGU A GUUAUAG
986 GUGUAGU A UUAAGGA
988 GUAGUAU U AGGCAAU
989 UAGUAUU A GGCAUUG
1007 CUGGCCU A GGCAUAA
1013 UAAGCAU A AUGGGAG
1024 GGAGAGU A CAGAGGU
1032 CAGAGGU A CACCGAG
1044 GAGGAUU C AAGAUUU
1050 UCAAGAU C UAUAUGA
1052 AAGAUCU A UAUGAUG
1054 GAUCUAU A UGAUGCA
1072 AAGGCAU A UGUGGAA
1085 AACAAU C AAAGAAA
1103 GUGUGAU U AACUACA
1104 UGUGAUU A ACUACAG
1108 AUUAACU A CAGUGUA
1115 ACAGUGU A CUAGACU
1118 GUGUACU A GACUUGA
1123 CUAGACU U GACAGCA
1139 AAGAAU C GAGGCUA
1146 AGAGGCU A UCAAAAC
1148 AGGCUAU C AAACAUC
1155 CAAACAU C AGCUUAA
1160 AUCAGCU U AAUCCAA
1161 UCAGCUU A AUCCAAA
1164 GCUUAUU C CAAAAGA
1173 AAAAGAU A AUGAUGU
1181 AUGAUGU A GAGCUUU
1187 UAGAGCU U UGAGUUA
1188 AGAGCUU U GAGUAAA
1193 UUUGAGU U AAUAAAA
1194 UUGAGUU A AAUAAAA

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Table 36: RSV (N) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
9	AUCUUUG CUGAUGAGGCCGAAAGGCCGAA AUUUGCC
21	UUGCUAA CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
23	CUUUGCU CUGAUGAGGCCGAAAGGCCGAA AGAGCCA
24	ACUUUGC CUGAUGAGGCCGAAAGGCCGAA AAGAGCC
32	UCAACUU CUGAUGAGGCCGAAAGGCCGAA ACUUUGC
37	AUCAUUC CUGAUGAGGCCGAAAGGCCGAA ACUUGAC
45	UUGAGUG CUGAUGAGGCCGAAAGGCCGAA AUCAUUC
50	CUUUGUU CUGAUGAGGCCGAAAGGCCGAA AGUGUAU
60	AGAAGUU CUGAUGAGGCCGAAAGGCCGAA AUCUUUG
65	AUGACAG CUGAUGAGGCCGAAAGGCCGAA AGUUGAU
66	GAUGACA CUGAUGAGGCCGAAAGGCCGAA AAGUUGA
70	GCUGGAU CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
73	UUUGCUG CUGAUGAGGCCGAAAGGCCGAA AUGACAG
82	GAUGGUG CUGAUGAGGCCGAAAGGCCGAA AUUUGCU
89	UCCGUUG CUGAUGAGGCCGAAAGGCCGAA AUGGUGU
108	UCAAUAC CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
111	GUUAUAA CUGAUGAGGCCGAAAGGCCGAA ACTAUUU
113	GAGUAUC CUGAUGAGGCCGAAAGGCCGAA AUACUAU
117	UUAAGAG CUGAUGAGGCCGAAAGGCCGAA AUCAAUA
120	UAAUUAG CUGAUGAGGCCGAAAGGCCGAA AGUAUCA
123	UCAUAUU CUGAUGAGGCCGAAAGGCCGAA AGGAGUA
126	ACAUCAU CUGAUGAGGCCGAAAGGCCGAA AUTAGGA
127	CACAUCA CUGAUGAGGCCGAAAGGCCGAA AAUUAAG
146	ACUUAUU CUGAUGAGGCCGAAAGGCCGAA AUGUGUU
150	CAUAACU CUGAUGAGGCCGAAAGGCCGAA AUUGAUG
154	GCCACAU CUGAUGAGGCCGAAAGGCCGAA ACUUAUU
155	UGCCACA CUGAUGAGGCCGAAAGGCCGAA AACUUAU
166	GAUUAUU CUGAUGAGGCCGAAAGGCCGAA ACAUGCC
167	UGAUUAA CUGAUGAGGCCGAAAGGCCGAA AACAUUC
169	UGUGAUU CUGAUGAGGCCGAAAGGCCGAA AUAACAU
170	CUUGGAU CUGAUGAGGCCGAAAGGCCGAA AAUAACA
173	CUUCUGU CUGAUGAGGCCGAAAGGCCGAA AUUAADA
186	UUAUGAU CUGAUGAGGCCGAAAGGCCGAA AGCAUCU
189	AAUUAUU CUGAUGAGGCCGAAAGGCCGAA AUUAGCA
192	GUGAAUU CUGAUGAGGCCGAAAGGCCGAA AUGAUUA
196	CCCAGUG CUGAUGAGGCCGAAAGGCCGAA AUUUUUG
197	ACCCAGU CUGAUGAGGCCGAAAGGCCGAA AAUUAUU
205	ACCUAUU CUGAUGAGGCCGAAAGGCCGAA ACCCAGU
206	UACCUAU CUGAUGAGGCCGAAAGGCCGAA AACCCAG
209	ACAUACC CUGAUGAGGCCGAAAGGCCGAA AUUAACC
213	UAUAACA CUGAUGAGGCCGAAAGGCCGAA ACCUAUU

217	CGCAUJU	CUGAUGAGGCGAAAGGCGGAA	ACAUACC
218	UCGCAUA	CUGAUGAGGCGAAAGGCGGAA	AACAUAAC
220	CADCGCA	CUGAUGAGGCGAAAGGCGGAA	AUAACAU
229	UAACCUA	CUGAUGAGGCGAAAGGCGGAA	ACADCGC
231	CCUAACC	CUGAUGAGGCGAAAGGCGGAA	AGACAUC
235	UCUCCU	CUGAUGAGGCGAAAGGCGGAA	ACCUAGA
236	CUCUCC	CUGAUGAGGCGAAAGGCGGAA	AACCUAG
254	GUUUUU	CUGAUGAGGCGAAAGGCGGAA	AUGGUGU
260	CUCUGAG	CUGAUGAGGCGAAAGGCGGAA	AUUUUUA
263	CAUCUCU	CUGAUGAGGCGAAAGGCGGAA	AGUADUU
277	UACAUGA	CUGAUGAGGCGAAAGGCGGAA	AUCCCGC
279	UUUACAU	CUGAUGAGGCGAAAGGCGGAA	AUAUCCC
284	UUGCUUU	CUGAUGAGGCGAAAGGCGGAA	ACAUGAU
299	UUACADC	CUGAUGAGGCGAAAGGCGGAA	ACUCCAU
305	GUGUGU	CUGAUGAGGCGAAAGGCGGAA	ACAUCUA
315	UCUUGAC	CUGAUGAGGCGAAAGGCGGAA	AUGUGUU
318	AUGCCUU	CUGAUGAGGCGAAAGGCGGAA	ACGAUGU
326	UUCCAU	CUGAUGAGGCGAAAGGCGGAA	AUGUCUU
327	UUCCAU	CUGAUGAGGCGAAAGGCGGAA	AADGUCU
346	CACUCCA	CUGAUGAGGCGAAAGGCGGAA	AUUUCAU
347	ACACUUC	CUGAUGAGGCGAAAGGCGGAA	AADUCCA
355	CAUGUU	CUGAUGAGGCGAAAGGCGGAA	ACACUUC
356	CCAADGU	CUGAUGAGGCGAAAGGCGGAA	AACACUU
361	GCUGCC	CUGAUGAGGCGAAAGGCGGAA	AUGUUAA
370	AGUUGUU	CUGAUGAGGCGAAAGGCGGAA	AGCUUGC
371	CAGUUGU	CUGAUGAGGCGAAAGGCGGAA	AAGCUUG
383	UGAUUG	CUGAUGAGGCGAAAGGCGGAA	AUUUCAG
384	UUGAUUU	CUGAUGAGGCGAAAGGCGGAA	AADUCCA
389	CAADGUU	CUGAUGAGGCGAAAGGCGGAA	AUUUGAA
395	CUAUCUC	CUGAUGAGGCGAAAGGCGGAA	AUGUUGA
401	UAGAUC	CUGAUGAGGCGAAAGGCGGAA	AUCUCAA
406	UUUUCUA	CUGAUGAGGCGAAAGGCGGAA	AUUUCUJ
408	GAUUUC	CUGAUGAGGCGAAAGGCGGAA	AGAUUCU
415	UUUGUAG	CUGAUGAGGCGAAAGGCGGAA	AUUUUUU
418	UUUUUG	CUGAUGAGGCGAAAGGCGGAA	AGGAUUU
431	UUUUUU	CUGAUGAGGCGAAAGGCGGAA	AGCAUUU
449	CUGGAGC	CUGAUGAGGCGAAAGGCGGAA	AUCCUC
453	UAUUCUG	CUGAUGAGGCGAAAGGCGGAA	AGCUACC
460	AUGCCUG	CUGAUGAGGCGAAAGGCGGAA	AUUCUGG
472	AUCAGGA	CUGAUGAGGCGAAAGGCGGAA	AGUCAUG
474	CAADUCAG	CUGAUGAGGCGAAAGGCGGAA	AGAGUCA
480	AUCCAC	CUGAUGAGGCGAAAGGCGGAA	AUCAGGA
491	AUAUAU	CUGAUGAGGCGAAAGGCGGAA	AUCAUCC
494	UACAUA	CUGAUGAGGCGAAAGGCGGAA	AUUAUCA
496	UADACAU	CUGAUGAGGCGAAAGGCGGAA	AUAUAUJ
497	CUAUACA	CUGAUGAGGCGAAAGGCGGAA	AADAUUA
501	GCUGCUA	CUGAUGAGGCGAAAGGCGGAA	ACAUAUJ
503	AUGCUGC	CUGAUGAGGCGAAAGGCGGAA	AUACADA
511	UAUUACU	CUGAUGAGGCGAAAGGCGGAA	AUGCUGC

512	UUUUUAC	CUGAUGAGGCCGAAAGGCCGAA	AADGCTG
515	UAGUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUUAUG
518	AUUUAGU	CUGAUGAGGCCGAAAGGCCGAA	AUUACTA
522	GCUAAUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUU
526	UGCUGCU	CUGAUGAGGCCGAAAGGCCGAA	AUUUAGU
527	CUGCUGC	CUGAUGAGGCCGAAAGGCCGAA	AUUUUAG
544	AAGACCA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGUC
549	GCUUUAA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGAU
551	CGGCGU	CUGAUGAGGCCGAAAGGCCGAA	AGACCAG
552	ACGGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGACCA
563	CUCUCCU	CUGAUGAGGCCGAAAGGCCGAA	AUCACGG
564	GCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AUCCAG
573	ACAUUU	CUGAUGAGGCCGAAAGGCCGAA	AGCUUUC
576	AGGACAU	CUGAUGAGGCCGAAAGGCCGAA	AUUAGCU
581	UUUUUAG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUU
584	CAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGGACAU
603	CCUUUGU	CUGAUGAGGCCGAAAGGCCGAA	ACGUUUC
604	GCCUUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUGUUU
613	GGGUAGU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUUU
614	UGGUUAG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCUU
617	CCUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUAAGC
629	UGUUGGC	CUGAUGAGGCCGAAAGGCCGAA	AUGUCCU
640	UUCAUAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGUU
641	CUUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGU
643	CACUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCU
652	UUUUUCA	CUGAUGAGGCCGAAAGGCCGAA	ACACUUC
653	GUUUUUC	CUGAUGAGGCCGAAAGGCCGAA	AACACUU
663	AAGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AUGUUUU
670	AUCUUA	CUGAUGAGGCCGAAAGGCCGAA	AGUGGGG
671	CAUCUUA	CUGAUGAGGCCGAAAGGCCGAA	AAGUGGG
672	ACAUCUA	CUGAUGAGGCCGAAAGGCCGAA	AAAGUGG
674	AAACADC	CUGAUGAGGCCGAAAGGCCGAA	AUAAAGU
680	GAACAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCUA
681	UGAACAA	CUGAUGAGGCCGAAAGGCCGAA	AACAUUU
682	AUGAACA	CUGAUGAGGCCGAAAGGCCGAA	AAACAUC
683	AADGAAC	CUGAUGAGGCCGAAAGGCCGAA	AAAACAU
686	CAAAAU	CUGAUGAGGCCGAAAGGCCGAA	ACAAAAA
687	CCAAAAU	CUGAUGAGGCCGAAAGGCCGAA	AACAAAA
690	AUACCAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAACA
691	UAUACCA	CUGAUGAGGCCGAAAGGCCGAA	AADGAAC
692	CUAUACC	CUGAUGAGGCCGAAAGGCCGAA	AAADGAA
696	UGUGCUA	CUGAUGAGGCCGAAAGGCCGAA	ACCAAAA
698	AUUGUGC	CUGAUGAGGCCGAAAGGCCGAA	AUACCAA
706	GGUAGAA	CUGAUGAGGCCGAAAGGCCGAA	AUUGUGC
708	CUGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUUGU
709	UCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AAGAUUG
711	CCUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAU
726	UCAACUC	CUGAUGAGGCCGAAAGGCCGAA	ACUGCCA
731	UCCCUUC	CUGAUGAGGCCGAAAGGCCGAA	ACUCUAC

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740	CUGCAA	CUGAUGAGGCGAAAGGCCGAA	ADCCCUU
741	CCUGCA	CUGAUGAGGCGAAAGGCCGAA	AAUCCCU
742	UCCUGCA	CUGAUGAGGCGAAAGGCCGAA	AAAUCCC
743	AUCCUGC	CUGAUGAGGCGAAAGGCCGAA	AAAAUCC
751	CAUAAAC	CUGAUGAGGCGAAAGGCCGAA	AUCCUGC
754	AUUCAU	CUGAUGAGGCGAAAGGCCGAA	ACAADCC
755	CADUCAU	CUGAUGAGGCGAAAGGCCGAA	AACAADC
756	GCADUCA	CUGAUGAGGCGAAAGGCCGAA	AAACAAU
766	UGCACCA	CUGAUGAGGCGAAAGGCCGAA	AGGCAUU
787	CCACCGU	CUGAUGAGGCGAAAGGCCGAA	ACAUCAC
788	CCACCG	CUGAUGAGGCGAAAGGCCGAA	AACAACA
800	UUGCUAA	CUGAUGAGGCGAAAGGCCGAA	ACUCCCC
802	UUUGCU	CUGAUGAGGCGAAAGGCCGAA	AGACUCC
803	AUUUUGC	CUGAUGAGGCGAAAGGCCGAA	AAGACUC
811	UUUAACU	CUGAUGAGGCGAAAGGCCGAA	AUUUUGC
815	UAUUUUU	CUGAUGAGGCGAAAGGCCGAA	ACUGAUU
816	AUAUUUU	CUGAUGAGGCGAAAGGCCGAA	AAUGAUU
822	AACAUA	CUGAUGAGGCGAAAGGCCGAA	AUUUUUA
824	CUAACAU	CUGAUGAGGCGAAAGGCCGAA	AUAUUUU
825	CUAACA	CUGAUGAGGCGAAAGGCCGAA	AAUAUUU
829	ADGUCCU	CUGAUGAGGCGAAAGGCCGAA	ACAUAUU
830	CADGUCC	CUGAUGAGGCGAAAGGCCGAA	AACAUA
840	UGCACAC	CUGAUGAGGCGAAAGGCCGAA	AGCAUGU
866	CCUCAAC	CUGAUGAGGCGAAAGGCCGAA	ACUUGUU
869	AAACCUC	CUGAUGAGGCGAAAGGCCGAA	ACAACUU
875	AUUCUA	CUGAUGAGGCGAAAGGCCGAA	ACCUCAA
876	UAUUCAU	CUGAUGAGGCGAAAGGCCGAA	AAACUCA
877	AUAUUA	CUGAUGAGGCGAAAGGCCGAA	AAACUUC
883	UUGGGCA	CUGAUGAGGCGAAAGGCCGAA	AUUCUA
895	ACCACCC	CUGAUGAGGCGAAAGGCCGAA	AUUUUUG
913	AUGGUAG	CUGAUGAGGCGAAAGGCCGAA	AUCCUGC
914	UAUGGUA	CUGAUGAGGCGAAAGGCCGAA	AAUCCUG
916	UAUAUGG	CUGAUGAGGCGAAAGGCCGAA	AGAAUCC
921	UUCAAUA	CUGAUGAGGCGAAAGGCCGAA	AUGGUAG
923	UGUCAA	CUGAUGAGGCGAAAGGCCGAA	AUAUGGU
925	GUUGUUC	CUGAUGAGGCGAAAGGCCGAA	AUAUAUG
943	UAUAUAU	CUGAUGAGGCGAAAGGCCGAA	AUGCUUU
946	AGAUAAU	CUGAUGAGGCGAAAGGCCGAA	AUGAUGC
947	AAGAUA	CUGAUGAGGCGAAAGGCCGAA	AAUGAUG
949	CAAAGAU	CUGAUGAGGCGAAAGGCCGAA	AUAUAUA
950	UCAAGA	CUGAUGAGGCGAAAGGCCGAA	AAUAUUG
952	AGUCAA	CUGAUGAGGCGAAAGGCCGAA	AUAUAUA
954	UGAGUCA	CUGAUGAGGCGAAAGGCCGAA	AGAUAAU
955	UUGAGUC	CUGAUGAGGCGAAAGGCCGAA	AAGAUAA
960	GGAAAUU	CUGAUGAGGCGAAAGGCCGAA	AGUCAAA
964	GUGAGGA	CUGAUGAGGCGAAAGGCCGAA	AUUGAGU
965	AGUGAGG	CUGAUGAGGCGAAAGGCCGAA	AAUUGAG
966	AAGUGAG	CUGAUGAGGCGAAAGGCCGAA	AAAUUGA
969	GAGAAGU	CUGAUGAGGCGAAAGGCCGAA	AGGAAAU

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973	ACUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUGAGG
974	CACUGGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUGAG
976	UACACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUG
983	CUAAUAC	CUGAUGAGGCCGAAAGGCCGAA	ACACUGG
986	UGOCUAA	CUGAUGAGGCCGAAAGGCCGAA	ACUACAC
988	AUUGCCU	CUGAUGAGGCCGAAAGGCCGAA	AUACUAC
989	CAUUGCC	CUGAUGAGGCCGAAAGGCCGAA	AAUACUA
1007	UUAUGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCAG
1013	CUCUCAU	CUGAUGAGGCCGAAAGGCCGAA	AUGCCUA
1024	ACCUUCG	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCC
1032	CUCGGUG	CUGAUGAGGCCGAAAGGCCGAA	ACCUUCG
1044	AGAUCUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCUCC
1050	UCAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGA
1052	CAUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AGAUCUU
1054	UGCADCA	CUGAUGAGGCCGAAAGGCCGAA	AUAGAUU
1072	UUCAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCCUU
1085	UUUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUU
1103	UGUAGUU	CUGAUGAGGCCGAAAGGCCGAA	AUCACAC
1104	CUGUAGU	CUGAUGAGGCCGAAAGGCCGAA	AADACAC
1108	UACACUG	CUGAUGAGGCCGAAAGGCCGAA	AGUUAUU
1115	AGUCUAG	CUGAUGAGGCCGAAAGGCCGAA	ACACUGU
1118	UCAAGUC	CUGAUGAGGCCGAAAGGCCGAA	AGUACAC
1123	UGCUGUC	CUGAUGAGGCCGAAAGGCCGAA	AGUCUAG
1139	UAGCCUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUCUU
1146	UGUUUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCCUCU
1148	GAUGUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAGCCU
1155	UUAAGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGUUUG
1160	UUGGAUU	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAU
1161	UUUGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGA
1164	UCUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGUAAGC
1173	ACAUCAU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUU
1181	AAAGCUC	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAU
1187	UAACTCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUCUA
1188	UUAACUC	CUGAUGAGGCCGAAAGGCCGAA	AAGCUCU
1193	UUUUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUCAA
1194	UUUUUAU	CUGAUGAGGCCGAAAGGCCGAA	AACUCAA

Table 87: RSV (IB) HP Ribozyme/Substrate Sequence

nt. Position	HP Ribozyme Sequence		Substrate
70	CUGUGNUG	AGAA GUCUUU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AAAGACU GAU GAUCACAG
91	CAGUGUAC	AGAA GUCUCA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UGAGACC GUU GUCACUUG
472	CAGGCUCC	AGAA GGAUUA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UAGUCCA GAU GAGGCCUG

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Table 38: RSV (N) HP Ribozyme/Substrate Sequence

nt. Position	Hairpin Ribozyme Sequence	Substrate
476	UCCCCACU AGA GAGAG ACCAGAGAAACACACGUGUGGUAACAUUACCUAGUA	CUCUCCU GAU UGUAGGAU
540	UAGACCCAG AGA GUCCCC ACCAGAGAAACACACGUGUGGUAACAUUACCUAGUA	GAGAGCA GAU CUGGUCUU
554	CUAAUCAC AGA GUAGA ACCAGAGAAACACACGUGUGGUAACAUUACCUAGUA	UCUUAUA GCC GUGAUAUG
636	UUCAUAGA AGA GUUGGC ACCAGAGAAACACACGUGUGGUAACAUUACCUAGUA	GCCAUUA GCU UCUAUAUA
998	CCUAGGCC AGA GCUAUG ACCAGAGAAACACACGUGUGGUAACAUUACCUAGUA	CAUUGCU GCU GCGCUAUG
1156	UUGGAUUA AGA GAUGUU ACCAGAGAAACACACGUGUGGUAACAUUACCUAGUA	AACAUUA GCU UUAUCCAA

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Table 39: Large-Scale Synthesis

Sequence	Activator [Added/Final] (min)	Amidite [Added/Final] (min)	Time*	% Full Length Product
A ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	85
A ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	89
(GGU) ₃ GGT	T [0.50/0.33]	[0.1/0.02]	15 m	78
(GGU) ₃ GGT	S [0.25/0.17]	[0.1/0.02]	15 m	81
C ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	90
C ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	97
U ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	80
U ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	85
A (36-mer)	T [0.50/0.33]	[0.1/0.02]	15/15m	21
A (36-mer)	S [0.25/0.17]	[0.1/0.02]	15/15 m	25
A (36-mer)	S [0.50/0.24]	[0.1/0.03]	15/15 m	25
A (36-mer)	S [0.50/0.18]	[0.1/0.05]	15/15 m	38
A (36-mer)	S [0.50/0.18]	[0.1/0.05]	10/5 m	42

*Where two coupling times are indicated the first refers to RNA coupling and the second to 2'-O-methyl coupling. S = 5-S-Ethyltetrazole, T = tetrazole activator. A is 5' -ucu ccA UCU GAU GAG GCC GAA AGG CCG AAA Auc ccu -3' where lowercase represents 2'-O-methylnucleotides.

Table 40: Base Deprotection

Sequence	Deprotection Reagent	Time (min)	T °C	% Full Length Product
iBu(GGU) ₄	NH ₄ OH/EtOH	16 h	55	62.5
	MA	10 m	65	62.7
	AMA	10 m	65	74.8
	MA	10 m	55	75.0
	AMA	10 m	55	77.2
iPrP(GGU) ₄	NH ₄ OH/EtOH	4 h	65	44.8
	MA	10 m	65	65.9
	AMA	10 m	65	59.8
	MA	10 m	55	61.3
	AMA	10 m	55	60.1
C ₉ U	NH ₄ OH/EtOH	4 h	65	75.2
	MA	10 m	65	79.1
	AMA	10 m	65	77.1
	MA	10 m	55	79.8
	AMA	10 m	55	75.5
A (36-mer)	NH ₄ OH/EtOH	4 h	65	22.7
	MA	10 m	65	28.9

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Table 41: 2'-O-Alkylsilyl Deprotection

Sequence	Deprotection Reagent	Time (min)	T °C	% Full Length Product
AgT	TBAF	24 h	20	84.5
	1.4 M HF	0.5 h	65	81.0
(GGU) ₄	TBAF	24 h	20	60.9
	1.4 M HF	0.5 h	65	67.8
C ₁₀	TBAF	24 h	20	86.2
	1.4 M HF	0.5 h	65	86.1
U ₁₀	TBAF	24 h	20	84.8
	1.4 M HF	0.5 h	65	84.5
B (36-mer)	TBAF	24 h	20	25.2
	1.4 M HF	1.5 h	65	30.6
A (36-mer)	TBAF	24 h	20	29.7
	1.4 M HF	1.5 h	65	30.4

B is 5'- UCU CCA UCU GAU GAG GCC GAA AGG CCG AAA AUC CCU

-3'.

Table 42: NMR Data for UC Dimers containing
Phosphorothioate Linkage

Synthesis #	Type	Delivery	Eq.	Wait	ASE (%)
3524	ribo	2 x 3 s	10.4	2 x 100 s	95.9
3525	ribo	2 x 3 s	10.4	2 x 75 s	92.6
3530	ribo	2 x 3 s	10.4	2 x 75 s	92.1
3526	ribo	1 x 5 s	08.6	1 x 300 s	100.0
3578	ribo	1 x 5 s	08.6	1 x 250 s	100.0
3529	ribo	1 x 5 s	08.6	1 x 150 s	73.7

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*Table 43: NMR Data for 15-mer RNA containing
Phosphorothioate Linkages*

Synthesis #	Type	Delivery	Eq.	Wait	ASE (%)
3581	ribo	1 x 5 s	08.6	1 x 250 s	99.6
3663	ribo	2 x 4 s	13.8	2 x 300 s	100.0
3582	2'-O-Me	1 x 5 s	08.6	1 x 250 s	99.7
3668	2'-O-Me	2 x 4 s	13.8	2 x 300 s	99.8
3632	2'-O-Me	1 x 5 s	08.6	1 x 300 s	99.8

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Table 44. Kinetics of Self-Processing *In Vitro*

Self-Processing Constructs	k (min ⁻¹)*
HH	1.16 ± 0.08
HDV	0.56 ± 0.15
HP(GC)	0.36 ± 0.06
HP(GU)	0.054 ± 0.003

* k represents the unimolecular rate constant for ribozyme self-cleavage determined from a non-linear, least-squares fit (KaleidaGraph, Synergy Software, Reeding, PA) to the equation:

$$(\text{Fraction Uncleaved Transcript}) = \frac{1}{kt} (1 - e^{-kt})$$

The equation describes the extent of ribozyme processing in the presense of ongoing transcription (Long & Uhlenbeck, 1994 *Proc. Natl. Acad. Sci. USA* 91, 6977) as a function of time (t) and the unimolecular rate constant for cleavage (k). Each value of k represents the average (± range) of values determined from two experiments.

Table 45

Entry	Modification	$t_{1/2}$ (m) Activity (t_A)	$t_{1/2}$ (m) Stability (t_S)	$\beta = t_S/t_A$ $\times 10$
1	U4 & U7 = U	1	0.1	1
2	U4 & U7 = 2'-O-Me-U	4	260	650
3	U4 = 2'-CH ₂ -U	6.5	120	180
4	U7 = 2'-CH ₂ -U	8	280	350
5	U4 & U7 = 2'-CH ₂ -U	9.5	120	130
6	U4 = 2'-CF ₂ -U	5	320	640
7	U7 = 2'-CF ₂ -U	4	220	550
8	U4 & U7 = 2'-CF ₂ -U	20	320	160
9	U4 = 2'-F-U	4	320	800
10	U7 = 2'-F-U	8	400	500
11	U4 & U7 = 2'-F-U	4	300	750
12	U4 = 2'-C-Allyl-U	3	>500	>1700
13	U7 = 2'-C-Allyl-U	3	220	730
14	U4 & U7 = 2'-C-Allyl-U	3	120	400
15	U4 = 2'-araF-U	5	>500	>1000
16	U7 = 2'-araF-U	4	350	875
17	U4 & U7 = 2'-araF-U	15	500	330
18	U4 = 2'-NH ₂ -U	10	500	500
19	U7 = 2'-NH ₂ -U	5	500	1000
20	U4 & U7 = 2'-NH ₂ -U	2	300	1500
21	U4 = dU	6	100	170
22	U4 & U7 = dU	4	240	600

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CLAIMSWhat is claimed is:

1. An enzymatic nucleic acid molecule which cleaves ICAM-1 mRNA, IL-5 mRNA, *rel A* mRNA, TNF- α mRNA sites shown in Table 23, 25, 27, or 28, CML associated mRNA selected from those identified as SEQ. ID NOS 1-25, or RSV mRNA or RSV genomic RNA in a region selected from the group consisting of 1C, 1B and N.
2. The enzymatic nucleic acid molecule of claim 1, the binding arms of which contain sequences complementary to any one of the sequences defined in any of those in Tables 2, 3, 6-9, 11, 13, 15-23, 27, 28, 31, 33, 34, 36, and 37.
3. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said nucleic acid molecule is in a hammerhead motif.
4. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said RNA molecule is in a hairpin, hepatitis delta virus, group 1 intron, *Neurospora* VS RNA or RNaseP RNA motif.
5. The enzymatic nucleic acid molecule of claim 1 or 2, comprising between 12 and 100 bases complementary to said mRNA or genomic RNA.
6. The enzymatic nucleic acid molecule of claim 5 comprising between 14 and 24 bases complementary to said mRNA or genomic RNA.
7. The enzymatic nucleic acid molecule of claim 1 or 2, comprising between 5 and 23 bases complementary to said mRNA or genomic RNA.
8. The enzymatic nucleic acid molecule of claim 7 comprising between 10 and 18 bases complementary to said mRNA or genomic RNA.
9. An enzymatic nucleic acid molecule consisting essentially of a sequence selected from the group of those shown in Tables 4-8, 10, 12, 14-16, 19-22, 24, 26-28, 30, 32, 34 and 36-38.
10. A mammalian cell including an enzymatic nucleic acid molecule of claims 1 or 2.

11. The cell of claim 10, wherein said cell is a human cell.
12. An expression vector including nucleic acid encoding an enzymatic nucleic acid molecule or multiple enzymatic molecules of claims 1 or 2 in a manner which allows expression of that enzymatic RNA molecule(s) within a mammalian cell.
13. A mammalian cell including an expression vector of claim 12.
14. The cell of claim 13, wherein said cell is a human cell.
15. A method for treatment of a pathological condition related to the mRNA level of ICAM-1, IL-5, *rel A*, TNF- α , or RSV by administering to a patient an enzymatic nucleic acid molecule of claim 1 or 2.
16. A method for treatment of a pathological condition related to the mRNA level of ICAM-1, IL-5, *rel A*, TNF- α , or RSV by administering to a patient an expression vector of claim 12.
17. The method of claims 15 or 16, wherein said patient is a human.
18. The method of claim 17 wherein said condition is selected from the group consisting of atherosclerosis, myocardial infraction, stroke, restenosis, heart diseases, cancer, rheumatoid arthritis, asthma, reperfusion injury, inflammatory or autoimmune disorders, transplant rejection, myocardial ischemia, stroke, psoriasis, Kawasaki disease, HIV and AIDS, and septic shock.
19. A nucleoside selected from the group consisting of 5'-C-alkylnucleoside, 2'-deoxy-2'-alkylnucleoside, nucleoside 5'-deoxy-5'-dihalo-methylphosphonate, nucleoside 5'-deoxy-5'-difluoro-methylphosphonate, nucleoside 3'-deoxy-3'-dihalo-methylphosphonate, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
20. A nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.

21. A nucleotide triphosphate comprising a nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
22. The 5'-C-alkylnucleoside of claim 19, wherein the sugar portion is in a talo configuration.
23. The 5'-C-alkylnucleoside of claim 19, wherein the sugar portion is in an allo configuration.
24. An oligonucleotide comprising a nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
25. An oligonucleotide comprising a moiety having the formula:
- wherein B is a nucleotide base or hydrogen; R1, R2 and R3 independently is selected from the group consisting of hydrogen, an alkyl group containing between 2 and 10 carbon atoms inclusive, an amine, an amino acid, and a peptide containing between 2 and 5 amino acids inclusive; and the zigzag lines are independently hydrogen or a bond.
26. An oligonucleotide comprising a 3'-amido or peptido group.
27. An oligonucleotide comprising a 5'-amido or peptido group.
28. The oligonucleotide of claim 24, 25, 26, or 27 having enzymatic activity.
29. Method for producing an enzymatic nucleic acid molecule having activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one nucleotide having an alkyl group at its 5'-position or 2'-position.

- 5 30. Method for conversion of a protected allo sugar to a protected talo sugar, comprising the step of contacting said protected allo sugar with triphenyl phosphine, diethylazodicarboxylate, p-nitrobenzoic acid under inversion causing conditions to provide said protected talo sugar.
- 10 31. Method for the synthesis of a nucleoside 5' or a 3'-dihalo-methylphosphonate comprising the step of condensing a difluoromethylphosphonate-containing sugar with a pyrimidine or purine under conditions suitable for forming a nucleoside 5'- or 3'-difluoromethylphosphonate.
- 15 32. The oligonucleotide of claim 3, wherein the normal hammerhead U4 and/or U7 positions are substituted with 2'-NH-amino acid.
33. A method for the synthesis of RNA comprising the step of providing 5-S-alkyltetrazole at a delivered 0.1-1.0 M concentration for the activation of a RNA amidite during a coupling step for less than or equal to 10 minutes.
- 20 34. A method for the synthesis of RNA comprising the step of providing 5-S-alkyltetrazole at 0.15-0.35 M effective, or final, concentration for the activation of a RNA amidite during a coupling step for less than or equal to 10 minutes.
- 25 35. A method for the deprotection of RNA comprising the step of providing alkylamine (MA) or NH₄OH/alkylamine (AMA) at between 60°C - 70°C for 5 to 15 minutes to remove any exocyclic amino protecting groups from protected RNA; wherein said alkyl is selected from the group consisting of methyl, ethyl, propyl and butyl.
- 30 36. A method for the deprotection of RNA alkylsilyl protecting groups comprising, contacting said groups with anhydrous triethylamine-hydrogen fluoride (aHF•TEA) trimethylamine or diisopropylethylamine at between 60 °C-70 °C for 0.25-24 h.
37. A method for the purification of an RNA molecule by passing said enzymatic RNA molecule over an HPLC column, wherein said HPCC column is an anion exchange chromatography column.

38. Method for one pot deprotection of RNA comprising, contacting a protected base with anhydrous methyl amine at between 60 °C-70 °C for at least 5 min, cooling the resulting mixture and contacting said mixture with TEA-3HF reagents under conditions which remove a protecting group of the 2'-hydroxyl position.
39. Method for synthesizing RNA containing a phosphorothioate linkage comprising the step of contacting 6-10 equivalents of 3H-1,2-benzodithiole-3-one 1,1-dioxide (Beaucage reagent) with the growing RNA chain for 5 seconds with a reaction time of at least 300 seconds.
40. Method of synthesizing RNA containing a phosphorothioate linkage comprising the step of achieving coupling with 5-S-ethyltetrazole or 5-S-methyltetrazole prior to sulfurization.
41. Method of claims 38, 39 or 40 wherein said RNA is enzymatically active.
42. Method for synthesizing 2'-deoxy-2'-amino-nucleoside phosphoramidite, comprising the step of protecting the 2'-amino group with a N-phtaloyl group.
43. The method of claim 42 wherein the said nucleoside lacks a base.
44. Method for synthesis of RNA comprising the step of: protecting the 2'-position of a nucleotide during said synthesis with a (trimethylsilyl)ethoxymethyl (SEM) group.
45. Method for covalently linking a SEM group to the 2'-position of a nucleotide, comprising the step of: contacting a nucleoside with an SEM-containing molecule under SEM bonding conditions.
46. The method of claim 45, wherein said conditions comprise dibutyltin oxide and tetrabutylammonium fluoride and SEM-Cl.
47. Method for removal of an SEM group from a nucleoside molecule or an oligonucleotide, comprising the step of: contacting said molecule or oligonucleotide with boron trifluoride etherate ($\text{BF}_3 \cdot \text{OEt}_2$) under SEM removing conditions.

48. The method of claim 57 wherein said (BF₃•OEt₂) is provided in acetonitrile.
49. One or more vectors comprising
- 5 a first nucleic acid sequence encoding a first ribozyme having intramolecular or intermolecular cleaving activity, said first ribozyme being selected from the group consisting of a hammerhead, hairpin, hepatitis delta virus, *Neurospora* VS RNA, Group I, and RNaseP motif;
- 10 and a second nucleic acid sequence encoding a second ribozyme having intermolecular cleaving activity, said Second ribozyme being selected from the group consisting of a hammerhead, hairpin, hepatitis delta virus, *Neurospora* VS RNA, Group I, and RNaseP motif and said second nucleic acid being flanked by other
- 15 nucleic acid sequences encoding RNA which is cleaved by said first ribozyme to release said second ribozyme from RNA encoded by said vector;
- wherein said first and second nucleic acid sequences may be on the same or separate nucleic acid molecules, and said vector encodes mRNA or comprises RNA which lacks secondary structure which
- 20 reduces release of said second ribozyme by more than 20%.
50. Cell comprising the vector of claim 49.
51. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions
- 25 between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs.
52. The RNA molecule of claim 51, wherein said molecule is transcribed by a RNA polymerase III based promoter system.
53. The RNA molecule of claim 51, wherein said molecule is transcribed
- 30 by a type 2 pol III promoter system.
54. The RNA molecule of claim 51, wherein said molecule is a chimeric tRNA.

55. The RNA molecule of claim 53, said RNA having A and B boxes of a type 2 pol III promoter separated by between 0 and 300 bases.
56. The RNA molecule of claim 53, wherein said desired RNA molecule is at the 3' end of said B box.
- 5 57. The RNA molecule of claim 53, wherein said desired RNA molecule is in between the said A and the B box.
58. The RNA molecule of claim 53, wherein said desired RNA molecule includes said B box.
- 10 59. The RNA molecule of claim 51, wherein said desired RNA molecule is selected from the group consisting of antisense RNA, decoy RNA, therapeutic editing RNA, enzymatic RNA, agonist RNA and antagonist RNA.
60. The RNA molecule of claim 51, wherein said 5' terminus is able to base-pair with at least 12 bases of said 3' region.
- 15 61. The RNA molecule of claim 51, wherein said 5' terminus is able to base-pair with at least 15 bases of said 3' region.
62. DNA vector encoding the RNA molecule of claim 51
63. The vector of claim 62, wherein said vector is derived from an AAV or adeno virus.
- 20 64. RNA vector encoding the RNA molecule of claim 51.
65. The vector of claim 64, wherein said vector is derived from an alpha virus or retro virus.
66. The vector of claim 62 wherein the portions of the vector encoding said RNA function as a RNA pol III promoter.
- 25 67. Cell comprising the vector of claim 62.
68. Cell comprising the vector of claim 53.
69. Cell comprising the RNA of claim 51.

70. Method to provide a desired RNA molecule in a cell, comprising introducing said molecule into said cell a RNA comprising a desired RNA molecule, having a 5' terminus able to base pair with at least 8 bases of a 3' region of said RNA molecule.
- 5 71. The method of claim 70, wherein said introducing comprises providing a vector encoding said RNA molecule.
72. Hammerhead ribozyme having 2 or 3 base pairs in stem II with an interconnecting loop of 4 or more bases between said base pairs.
- 10 73. Hairpin ribozyme lacking a substrate moiety, comprising at least six bases in helix 2 and able to base-pair with a separate substrate RNA, wherein the said ribozyme comprises one or more bases 3' of helix 3 able to base-pair with the said substrate RNA to form a helix 5 and wherein the said ribozyme can cleave and/or ligate said separate RNA(s) *in trans*.
- 15 74. The ribozyme of claim 73, wherein said ribozyme comprises six bases in helix 2.
75. The ribozyme of claim 73, having the structure of Fig. 3, wherein each N and N' is independently any base and each dash may represent a hydrogen bond, r is 1-20, q is 2-20, o is 0 - 20, n is 1 - 4, and m is 1 - 20.
- 20 76. Method for increasing the activity of a hairpin ribozyme by providing one or more bases 3' of helix 3 able to base-pair with a substrate RNA to form a helix 5.
77. Trans-cleaving Hairpin ribozyme comprising at least 6 base pairs in helix 2 lacking a substrate RNA moiety.
- 25 78. Trans-ligating Hairpin ribozyme comprising at least 6 base pairs in helix 2 lacking a substrate RNA moiety.
79. The ribozyme of claim 73 having the structure of Fig. 73.
80. The ribozyme of claim 73 having the structure of Fig. 74.
- 30 81. A cell including the ribozyme of any of claims 73-80.

82. An expression vector comprising nucleic acid encoding the ribozyme of any of claims 73-80, in a manner which allows expression of that ribozyme within a cell.
83. A cell including an expression vector of claim 82.
- 5 84. Method for altering in vivo the nucleotide base sequence of a naturally occurring mutant nucleic acid molecule, comprising the steps of:
- 10 contacting said nucleic acid molecule in vivo with an oligonucleotide or peptide nucleic acid able to form a duplex or triplex molecule with said nucleic acid molecule, wherein formation of said duplex or triplex molecule directly, or after nucleic acid repair *in vivo*, causes at least one base in said nucleic acid molecule to be chemically modified to functionally alter the nucleotide base sequence of said nucleic acid sequence.
- 15 85. The method of claim 84, wherein said oligonucleotide is of a length sufficient to activate dsRNA deaminase in vivo to cause conversion of an adenine base to inosine in an RNA molecule.
- 20 86. The method of claim 84, wherein said oligonucleotide comprises an enzymatic nucleic acid molecule which is active to chemically modify a base.
87. The method claim 84, wherein said nucleic acid molecule is DNA or RNA.
88. The method of claim 84, wherein said oligonucleotide comprises a chemical mutagen.
- 25 89. The method of claim 88, wherein said mutagen is nitrous acid.
90. The method of claim 84 wherein said oligonucleotide causes deamination of 5-methylcytosine to thymidine, cytosine to uracil, or adenine to inosine, or methylation of cytosine to 5-methylcytosine.
- 30 91. The method of claim 84, wherein an endogenous mammalian editing system is co-opted to cause said chemical modification.

92. Method for introduction of enzymatic nucleic acid into a cell or tissue, comprising the steps of;

5 providing a complex of a first nucleic acid molecule encoding said enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

10 and contacting said complex with said cell or tissue under conditions in which said enzymatic nucleic acid molecule is produced in said cell or tissue.

93. Method for introduction of a desired nucleic acid into a cell or tissue, comprising the steps of;

15 providing a complex of a first nucleic acid molecule encoding said desired nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said first nucleic acid molecule lacks a promoter region and said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

20 and contacting said complex with said cell or tissue under conditions in which said desired acid molecule is produced in said cell or tissue.

94. Method for introduction of a desired nucleic acid into a cell or tissue, comprising the steps of;

30 providing a complex of a first nucleic acid molecule encoding said enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired

structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

5 and wherein said second nucleic acid further comprises a localization factor;

and contacting said complex with said cell or tissue under conditions in which said desired nucleic acid molecule is produced in said cell or tissue.

- 10 95. Complex of a first nucleic acid molecule encoding an enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule;
- 15 wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.
- 20 96. Complex of a first nucleic acid molecule encoding a desired nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said first nucleic acid molecule lacks a promoter region and said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.
- 25
- 30 97. Complex of a first nucleic acid molecule encoding an enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said

first nucleic acid under said conditions, and wherein said second nucleic acid further comprises a localization factor.

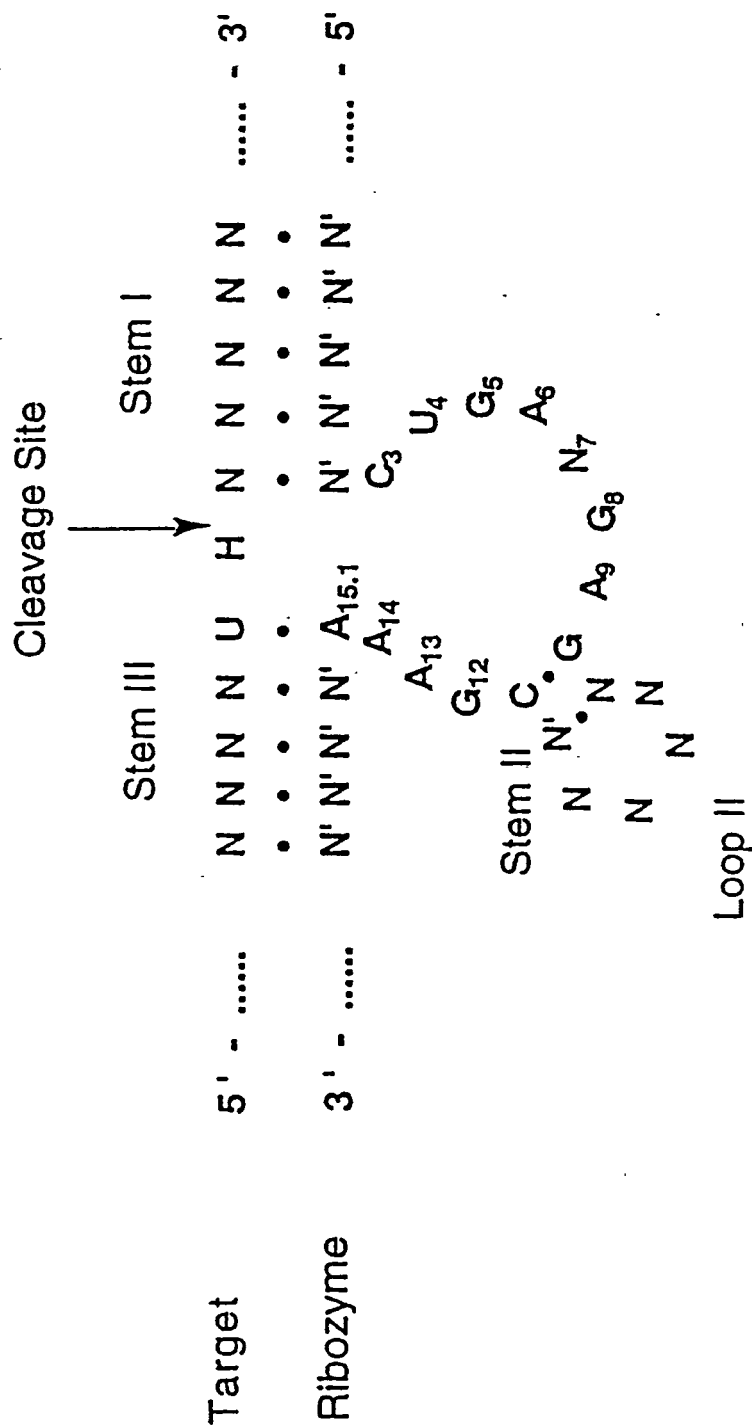
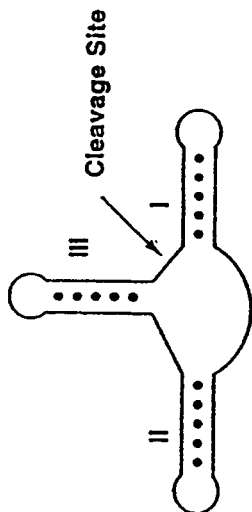


FIG. 1.

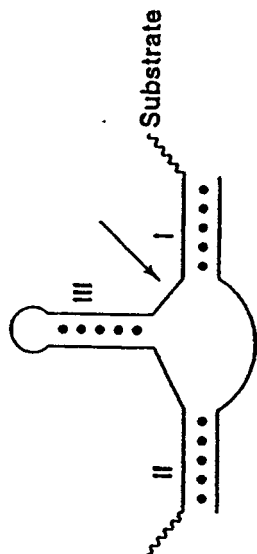
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FIG. 2a.



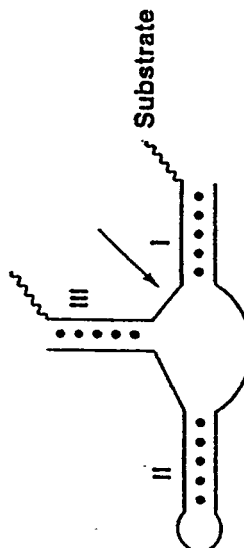
a

FIG. 2b.



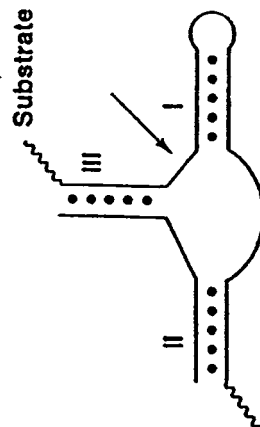
b

FIG. 2c.



c

FIG. 2d.



d

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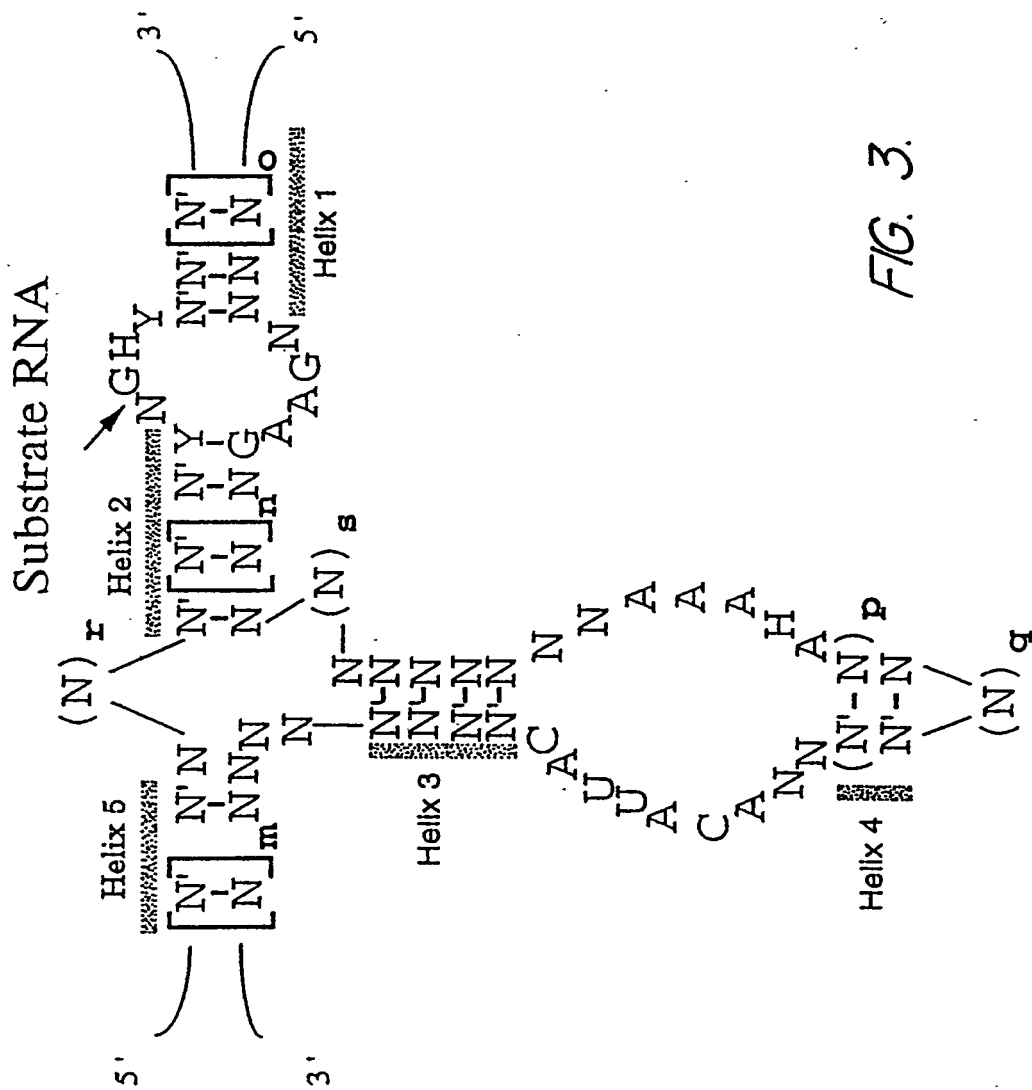
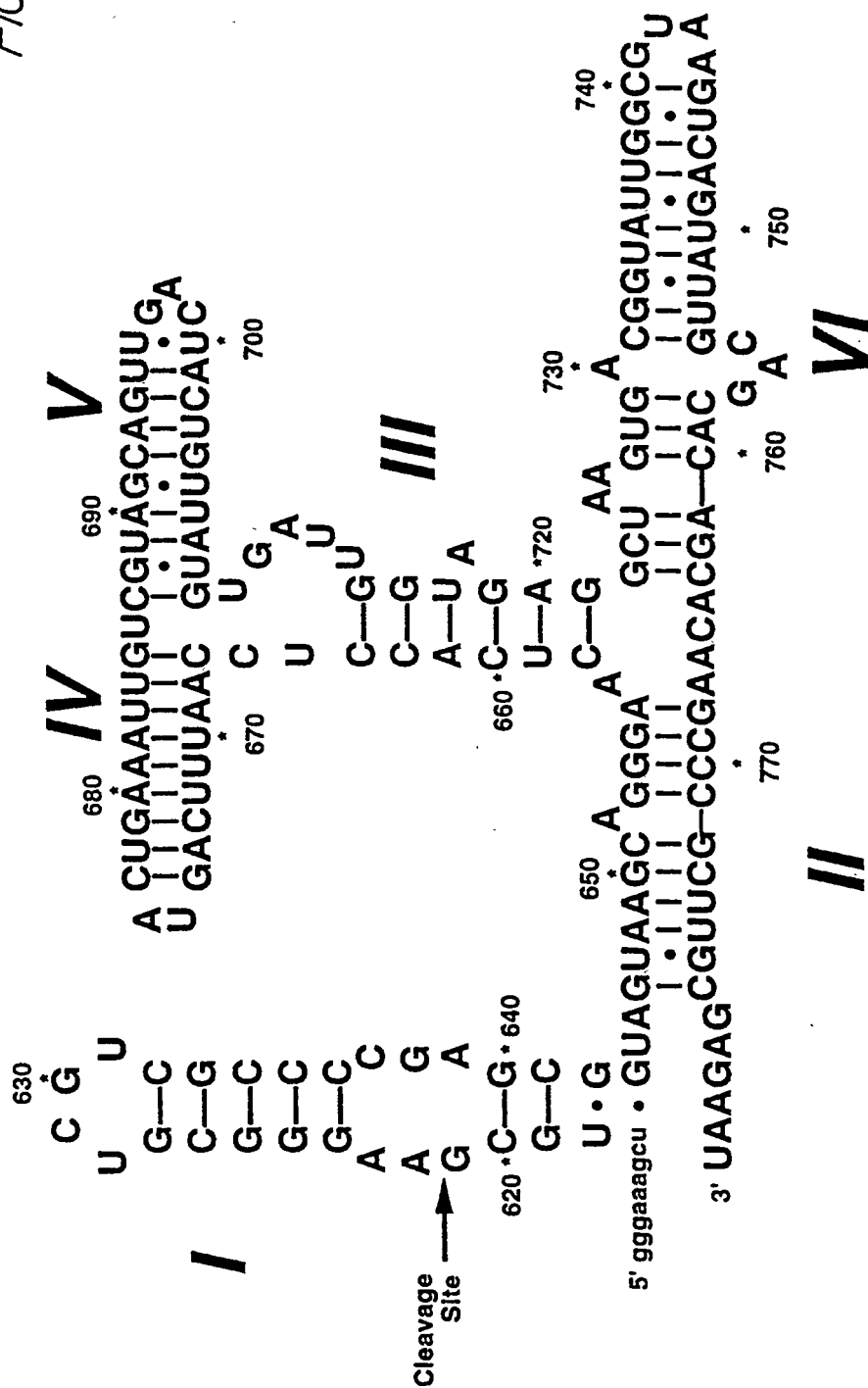


FIG. 3.

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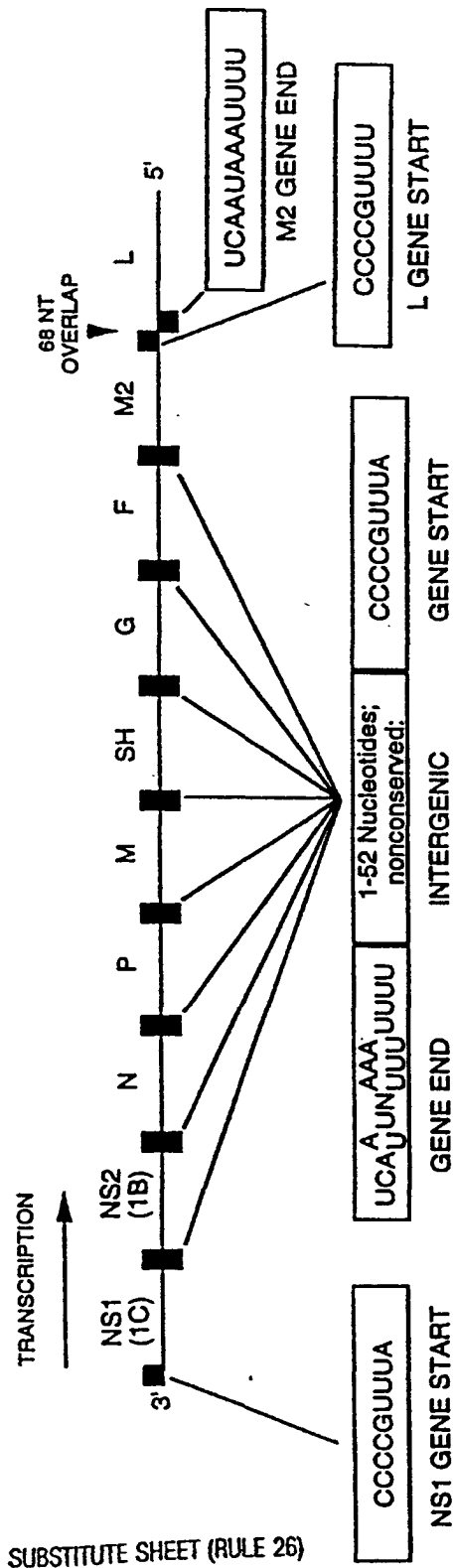
FIG. 5.



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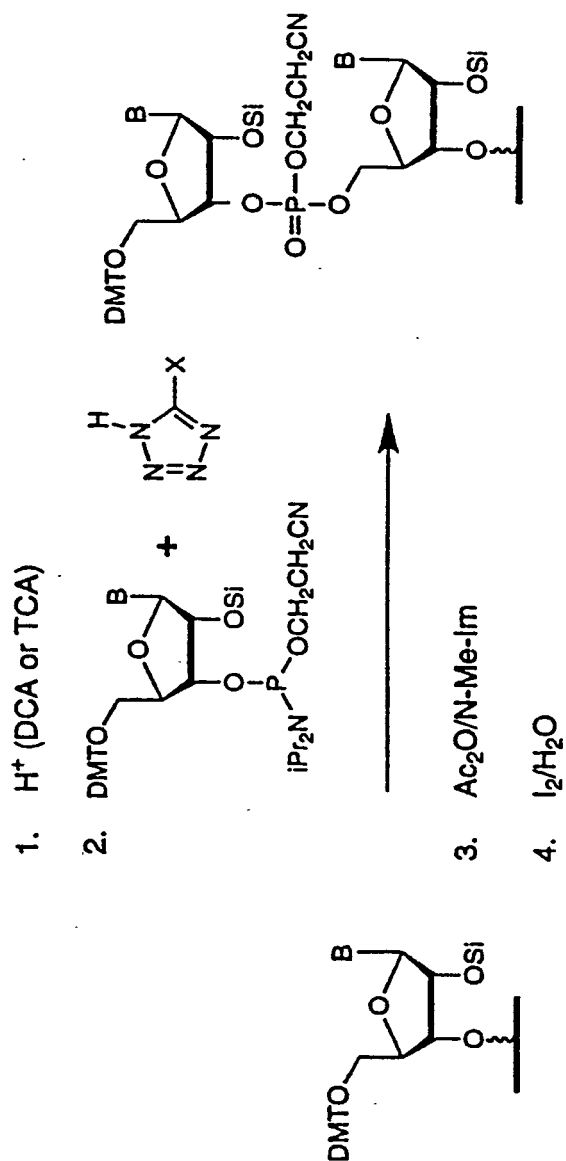
FIG. 6.



Adapted from Virology, Second Edition, Edited by B.N. Fields, 1990.

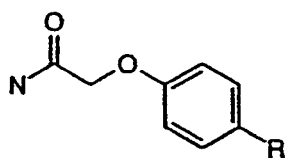
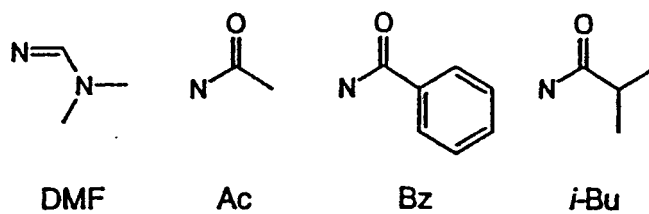
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FIG. 7.



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*FIG. 8.*

R = H = PAC

R = tBu = TAC

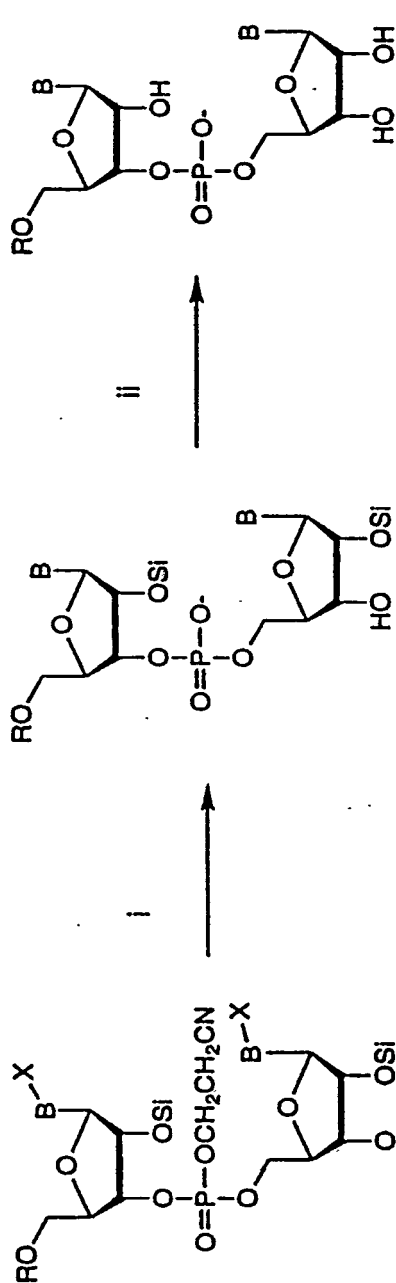
R = iPr = iPPAC

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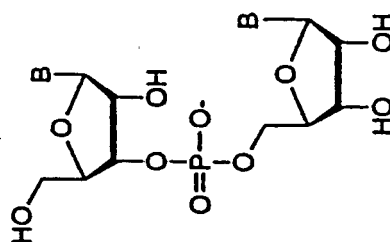
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FIG. 9.



- i MA or AMA, 30 m @ 65 °C
- ii anhydrous TEA·HF, 30 m @ 65 °C
- iii H⁺



R = H or DMT or other hydroxyl protection

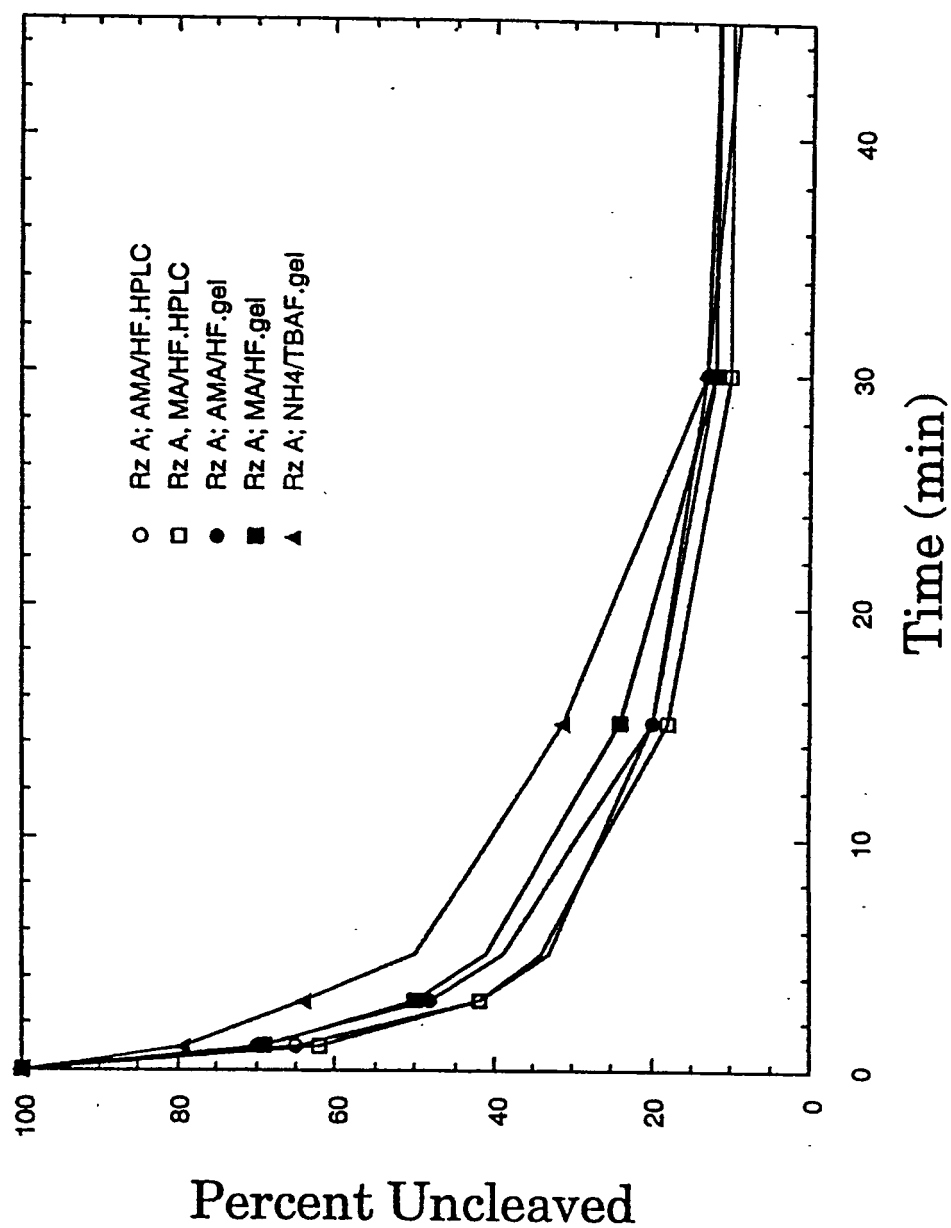
X = Exocyclic Amino protection

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FIG. 10.

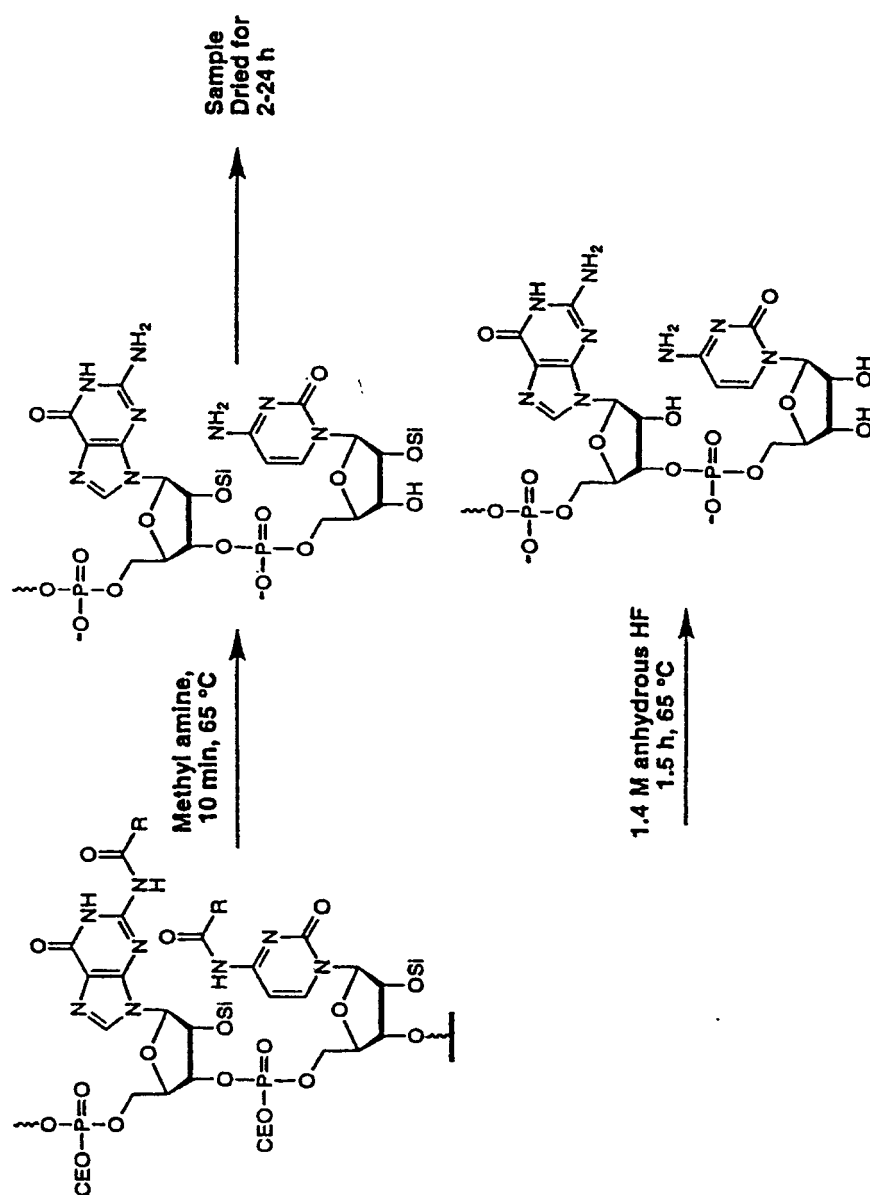


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FIG. 11.



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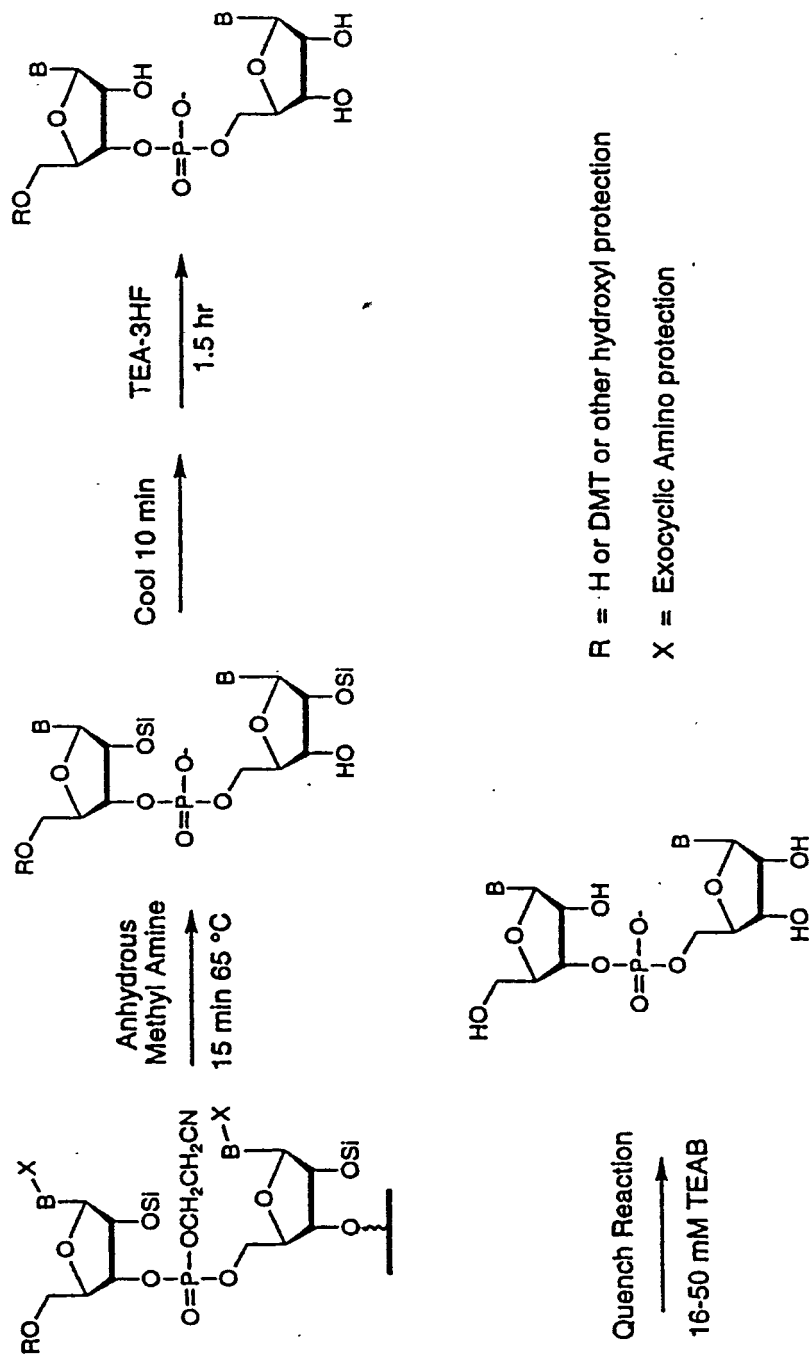
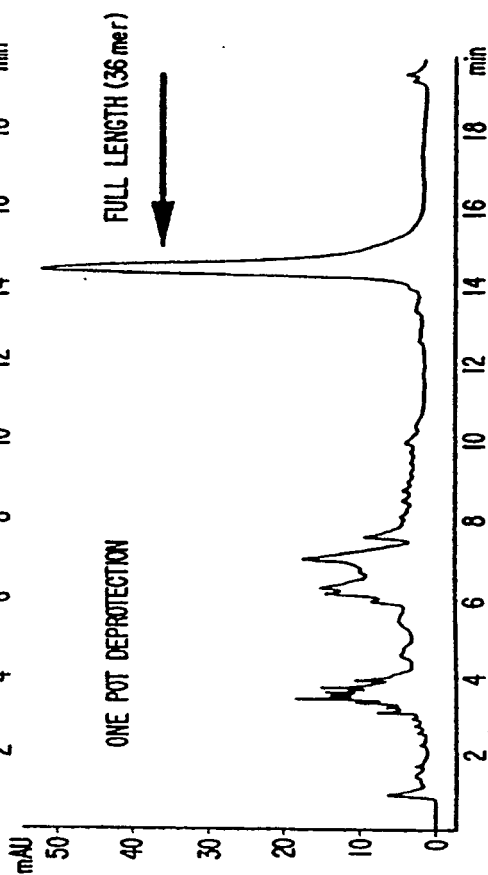
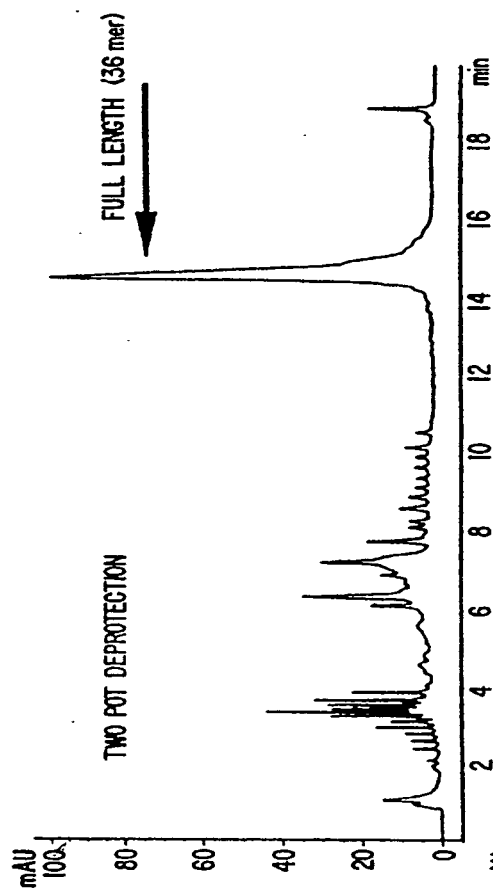


FIG. 12.

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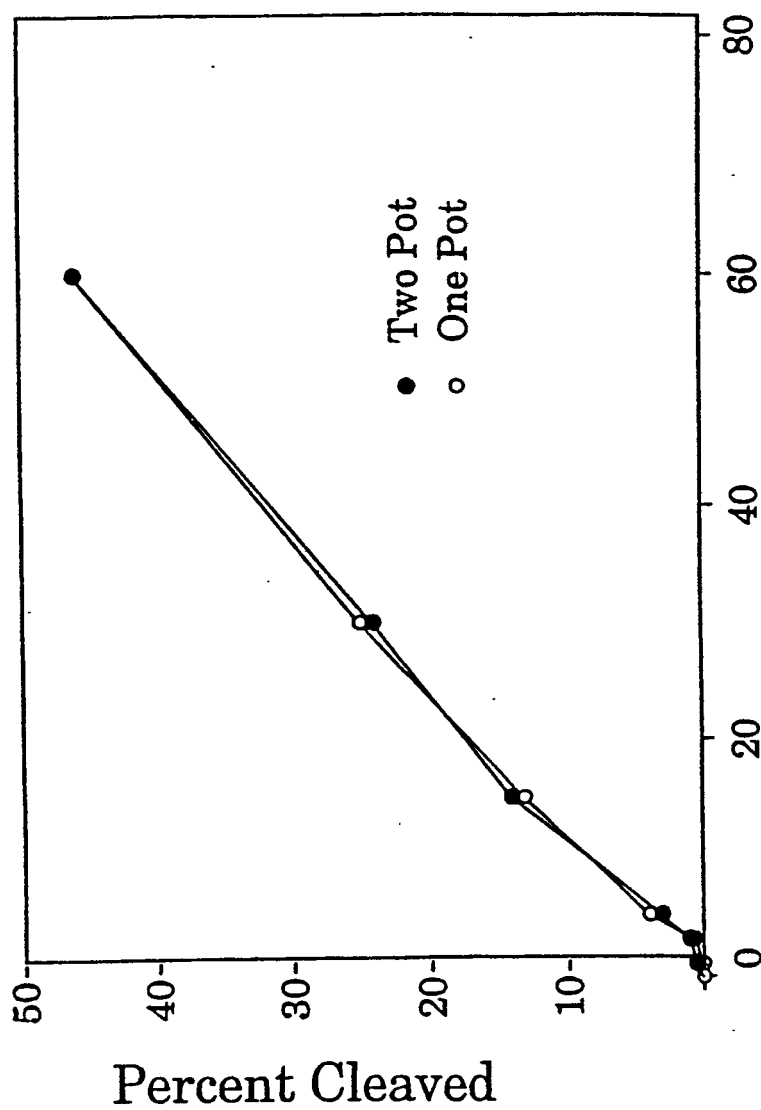
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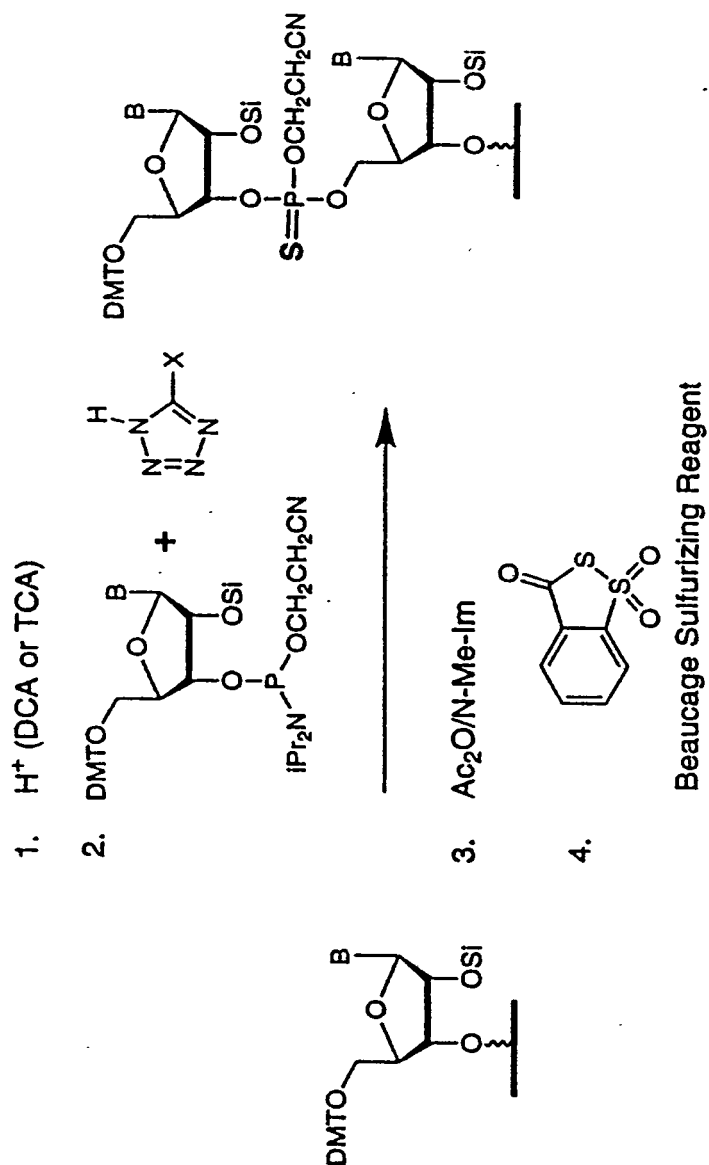
Time (min)
FIG. 14.

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FIG. 15.



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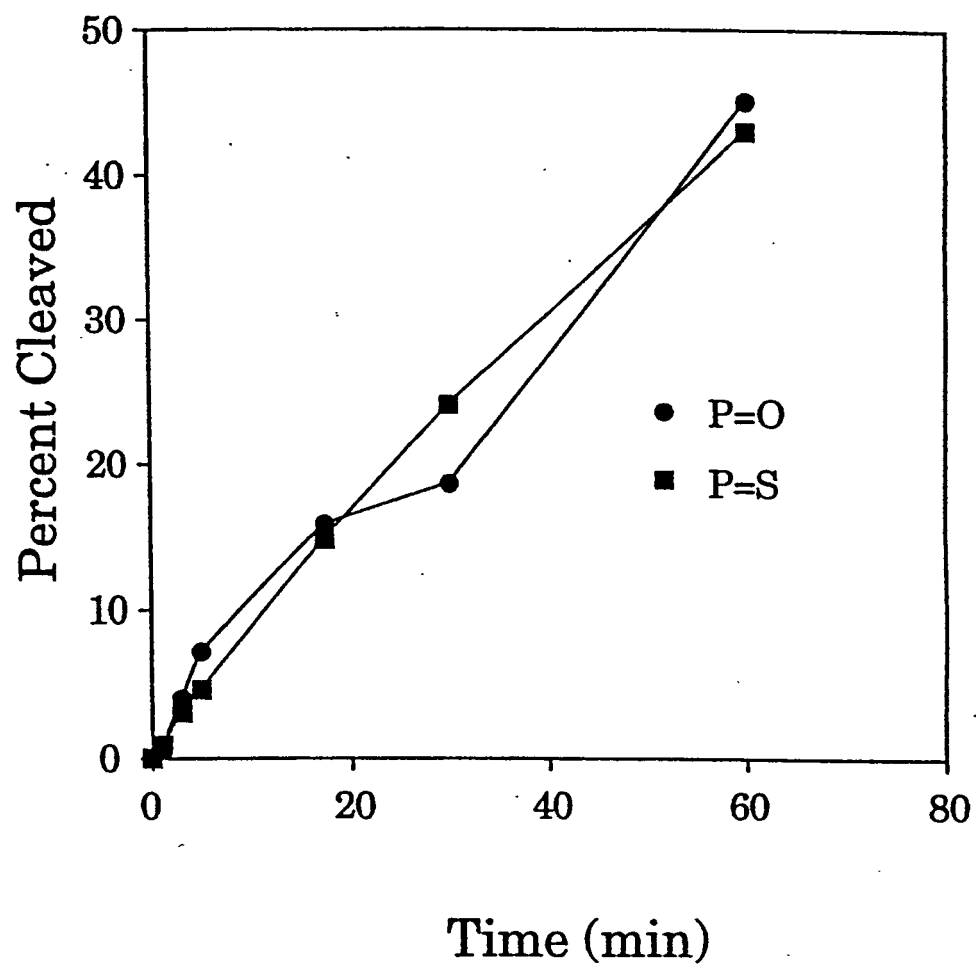
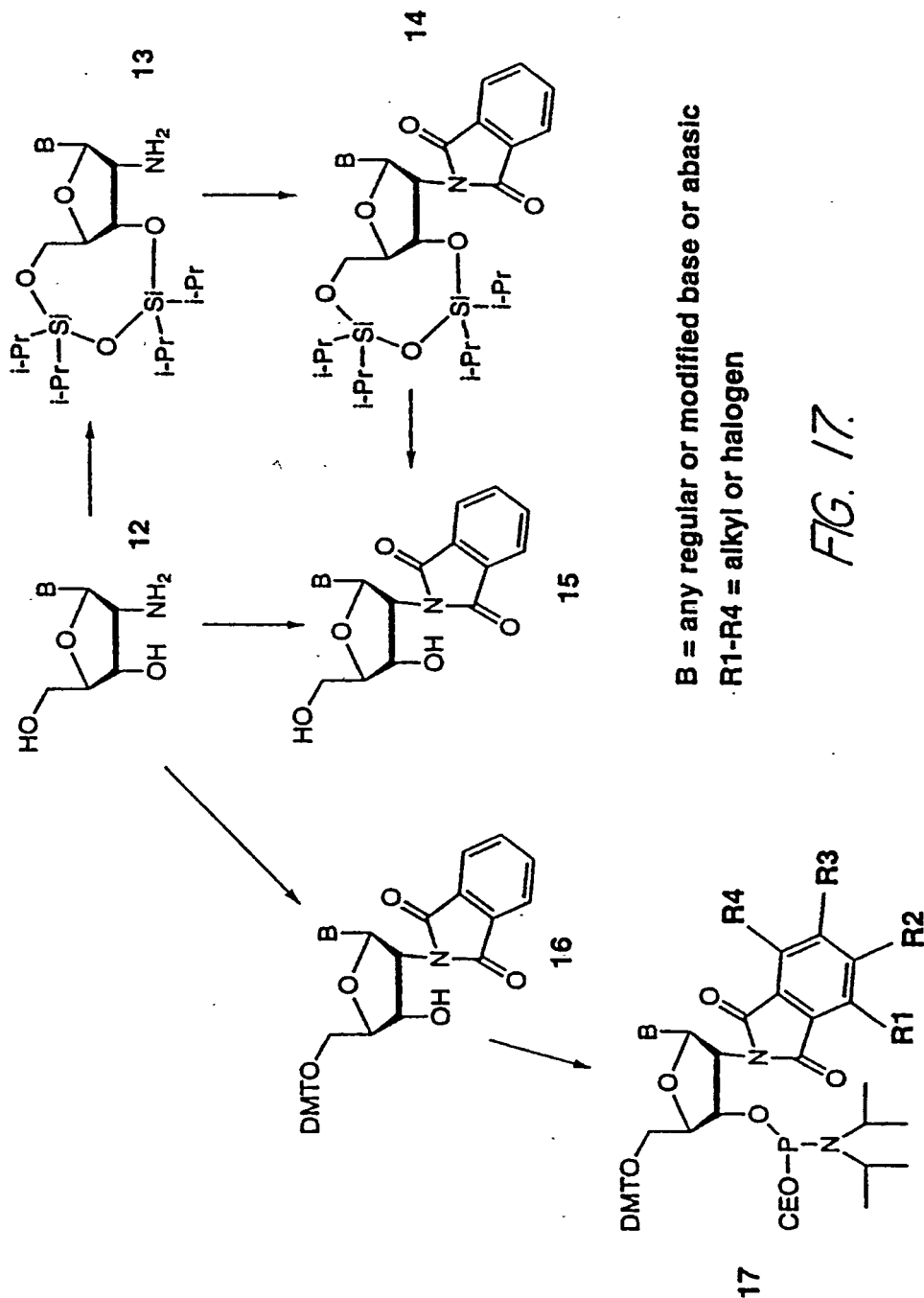


FIG. 16.

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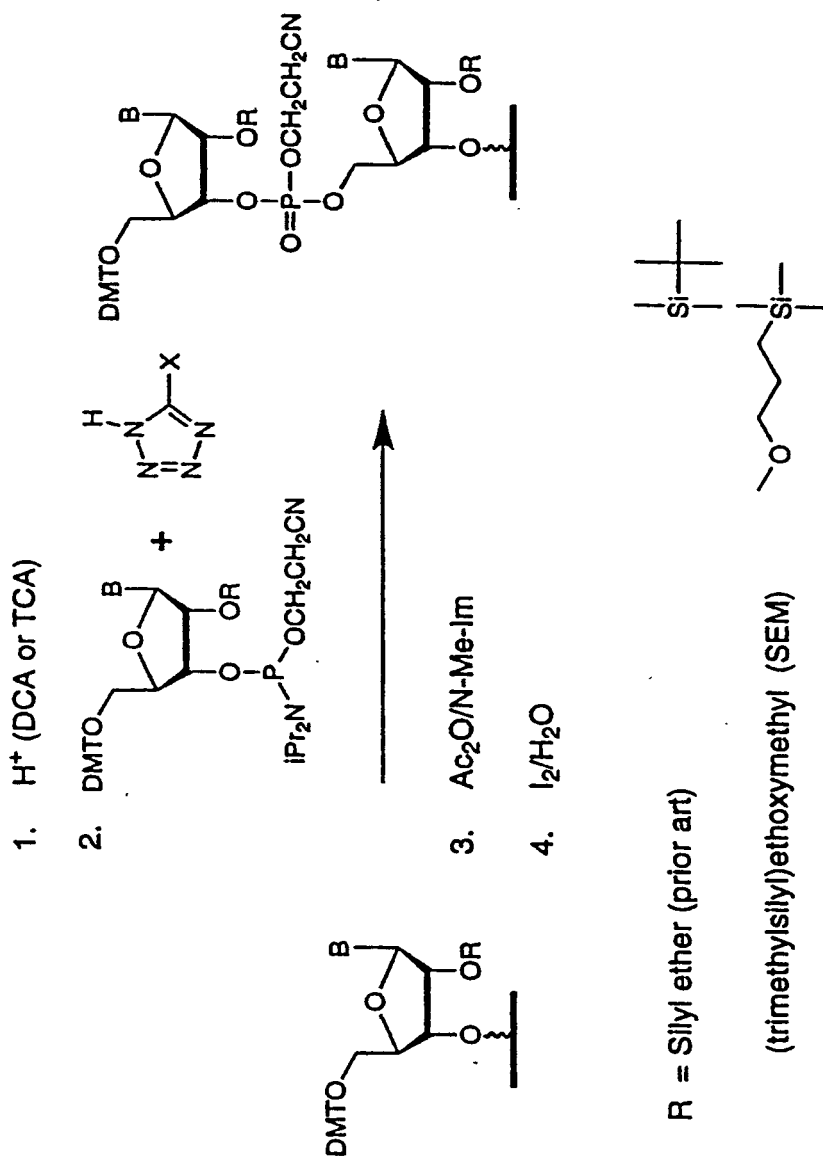


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FIG. 18.

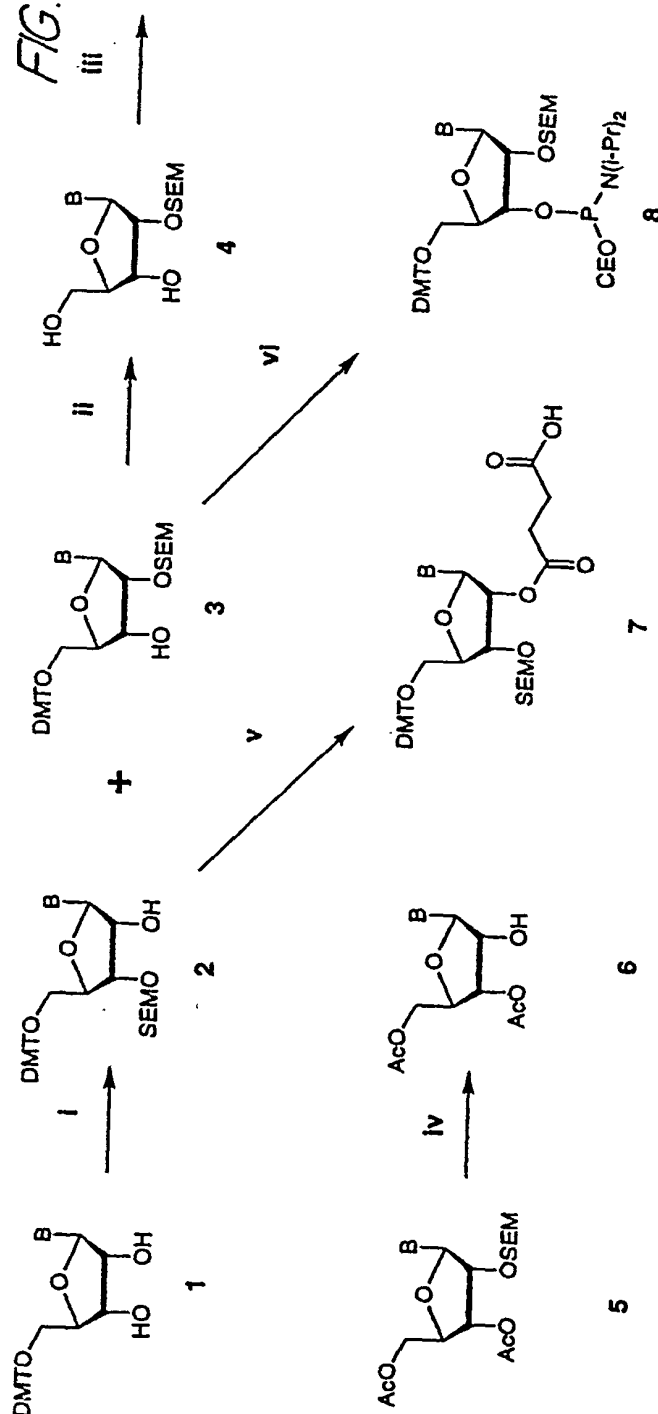


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FIG. 19.

i) = $\text{SnBu}_2\text{O/SEM-Cl}$ ii) = H^+ iii) = Ac_2O iv) = $\text{BF}_3\cdot\text{OEt}_2$

v) = Succinic Anhydride

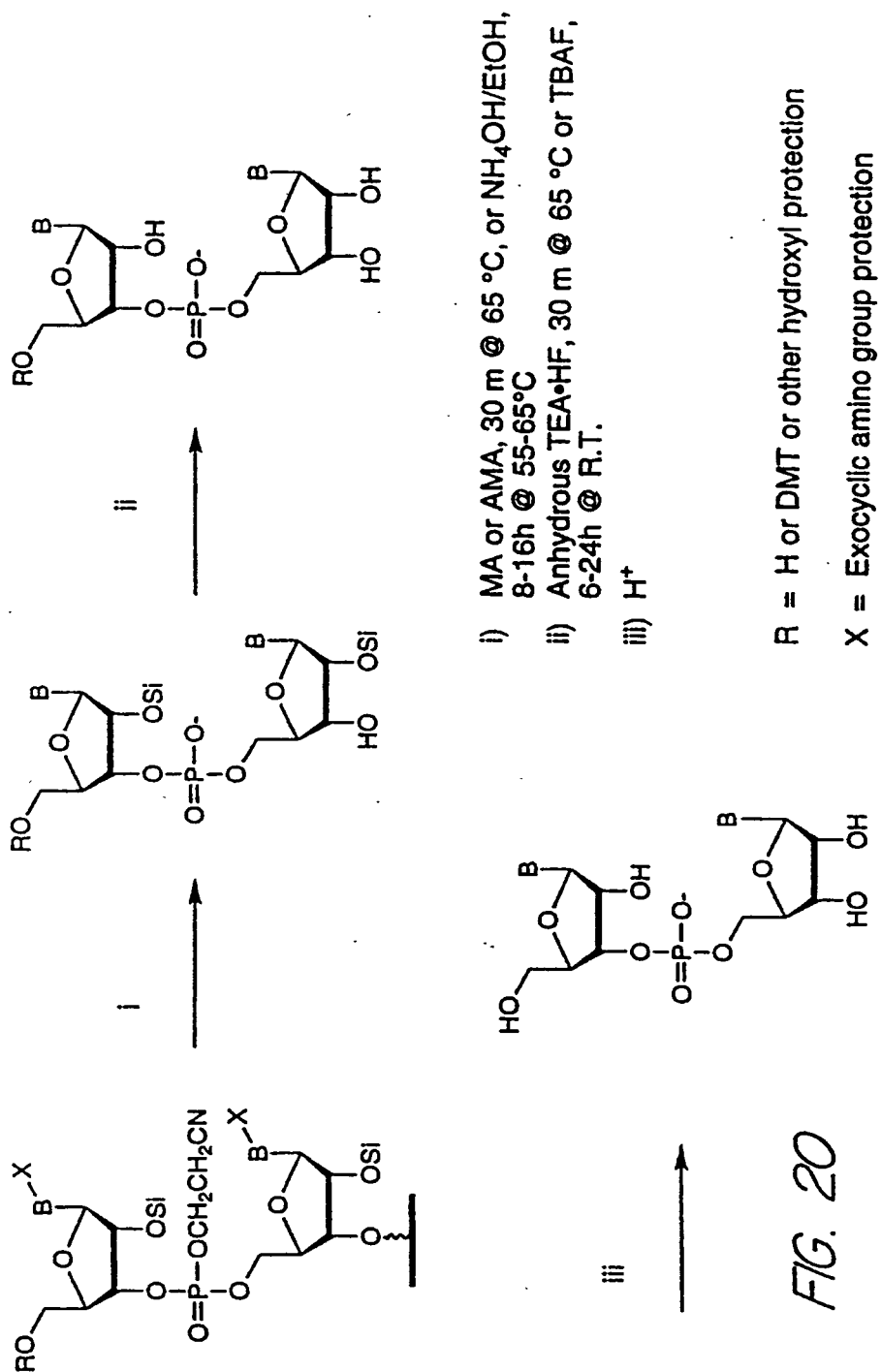
vi) = $\text{P(OCE)(N-IPr}_2\text{)Cl}$

B = Protected A, C, G, U, T, 2AP, I, DIAP, P etc.

SEM = (trimethylsilyl)ethoxymethyl

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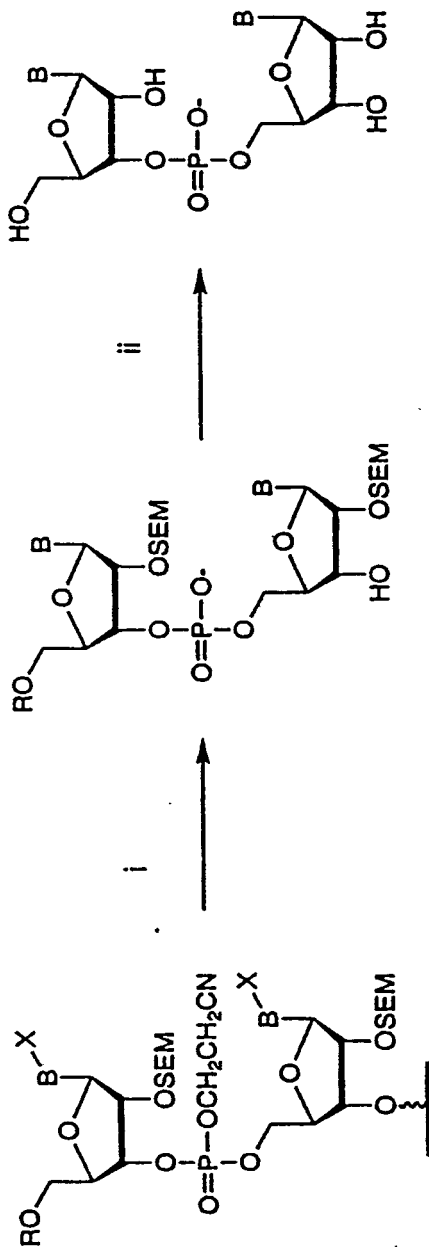
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FIG. 21.

i) MA or AMA, 30 m @ 65 °C or NH₄OH or NH₄OH/EtOH, 8-16h @ 55-65°Cii) BF₃•OEt₂

SEM = (trimethylsilyl)ethoxymethyl

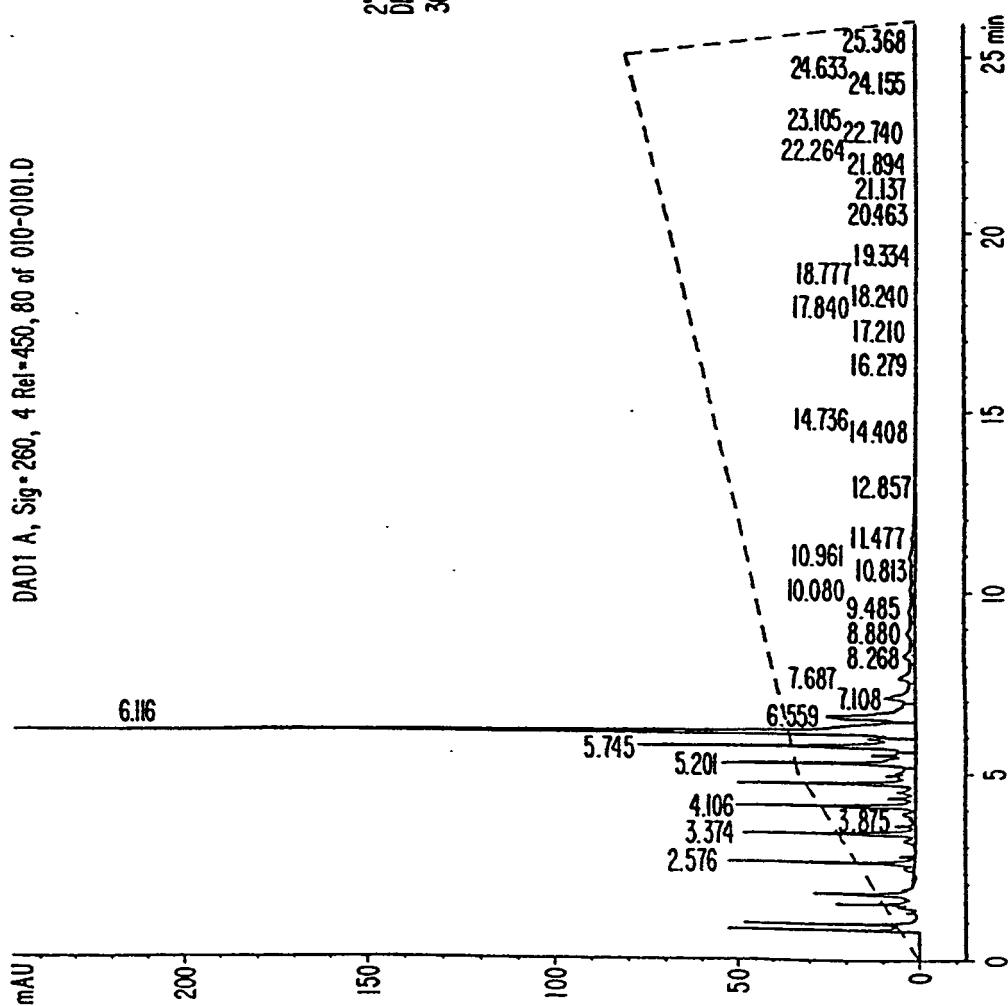
R = H or DMT or other hydroxyl protection

X = Exocyclic amino group protection

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FIG. 22.

2'-O-SEM PROTECTED U 10-mer
DEPROTECTED WITH $\text{BF}_3 \cdot \text{OEt}_2$
30m, 3eq./nucleotide

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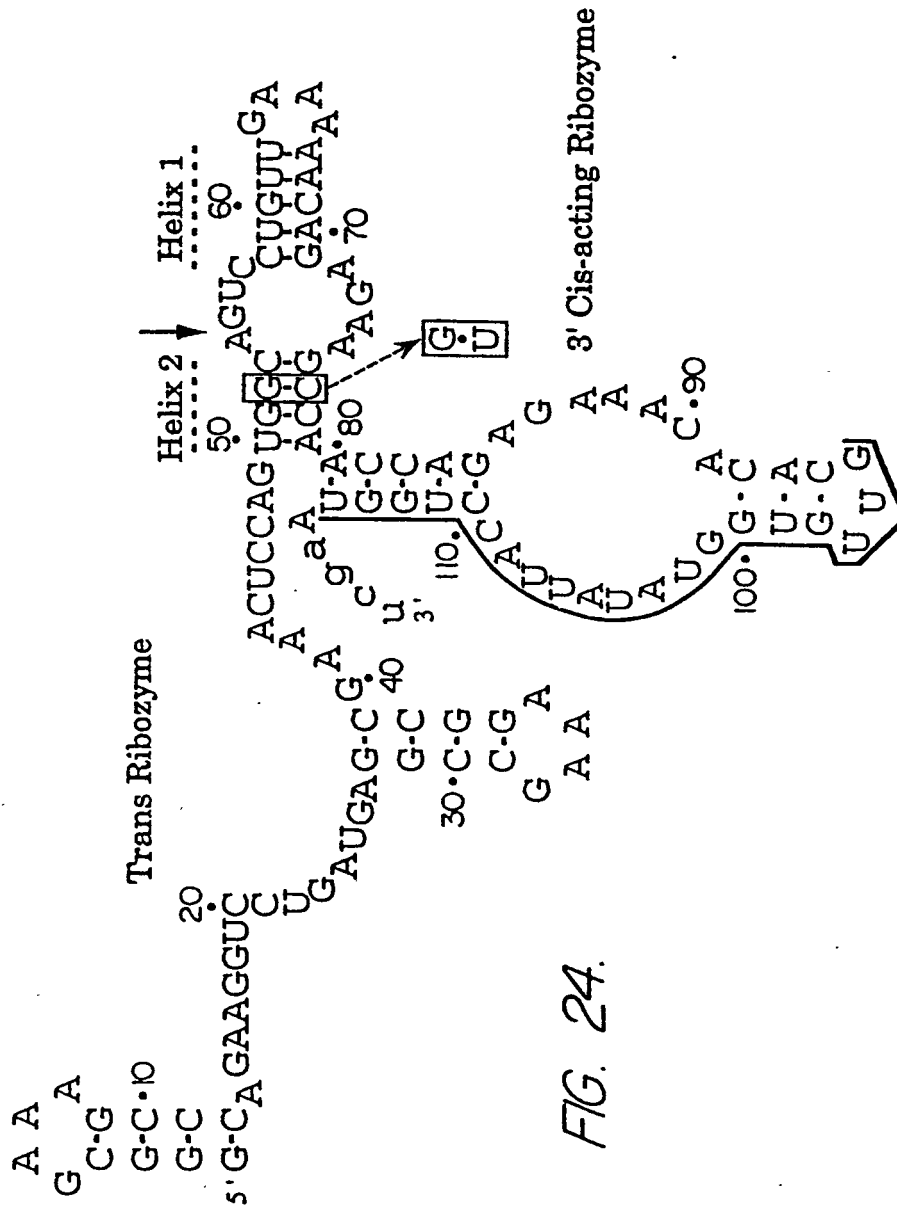


FIG. 24.

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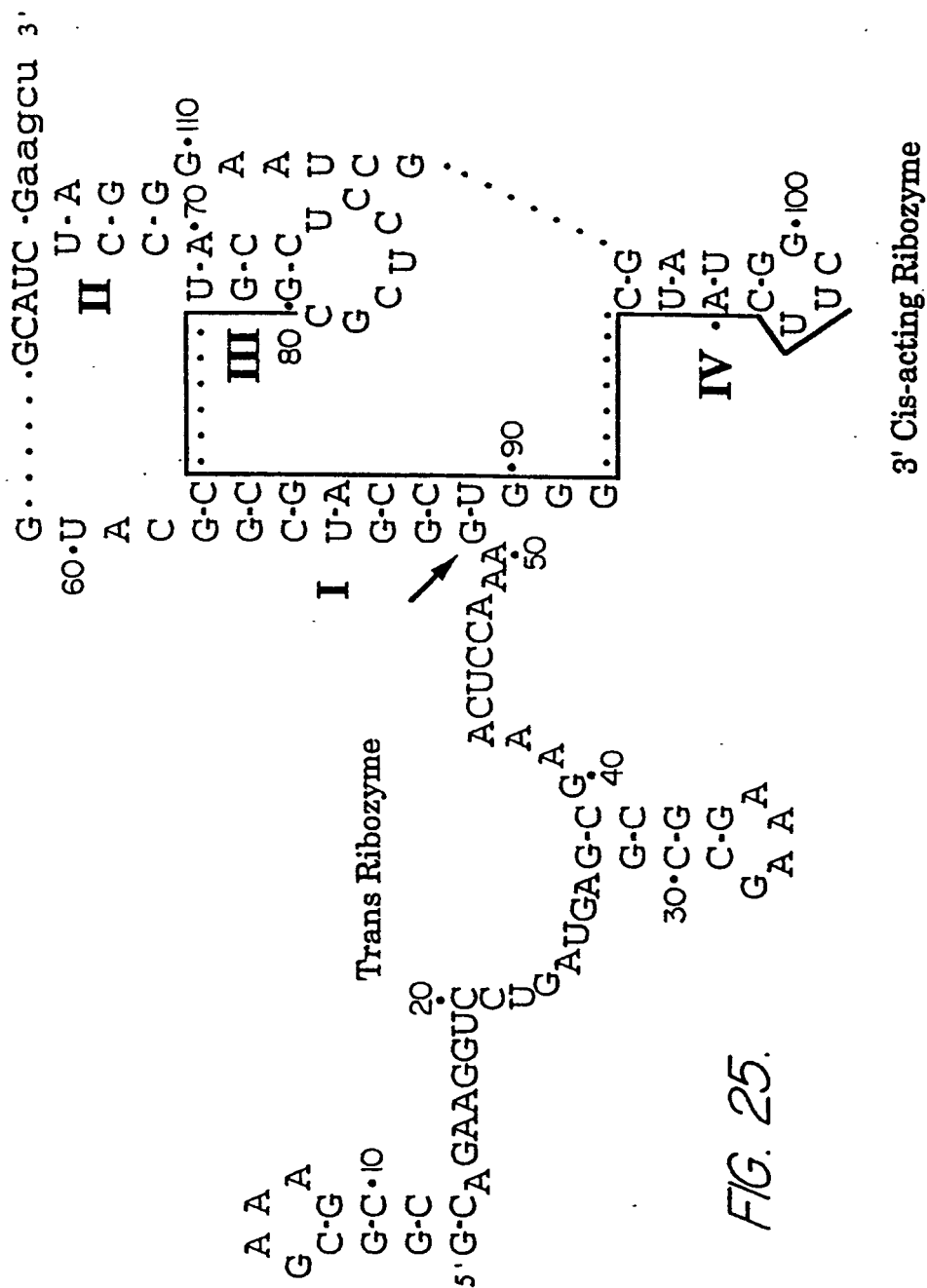
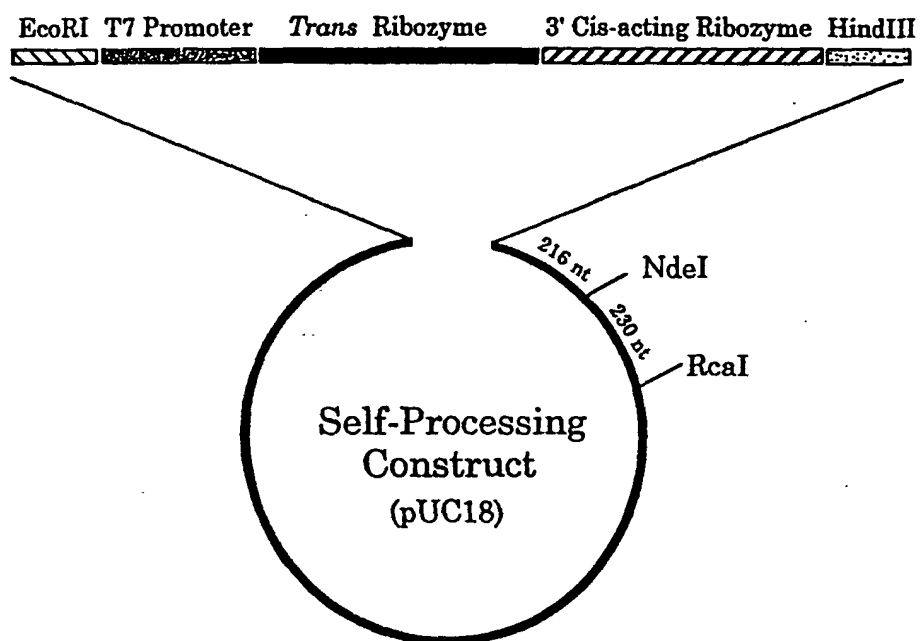


FIG. 25.

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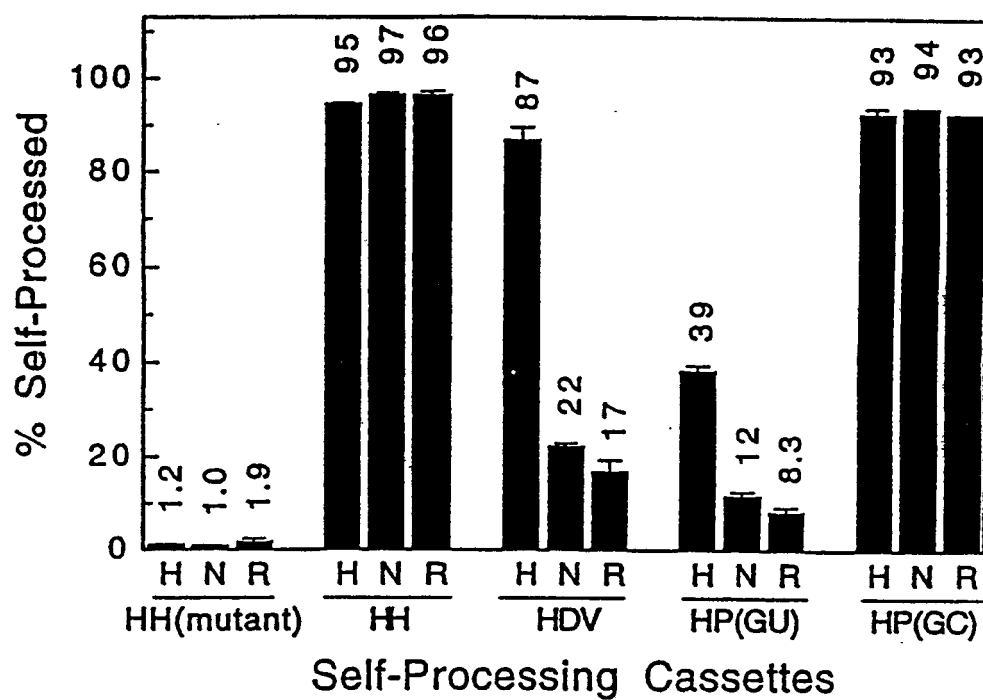
FIG. 26.



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FIG. 27.



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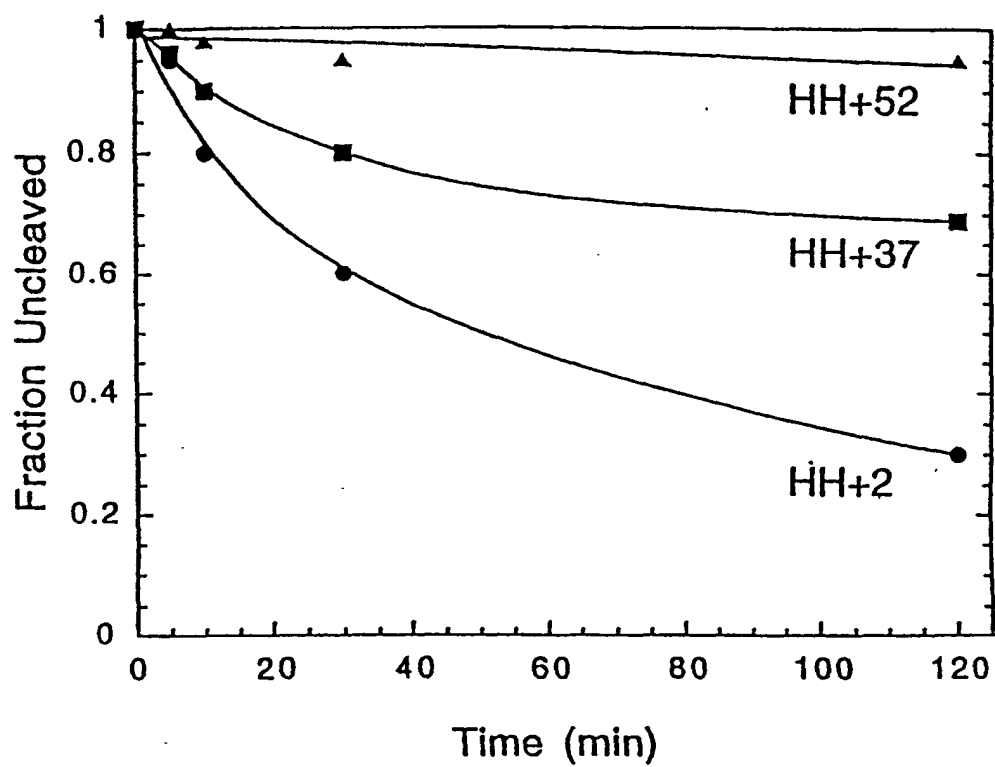


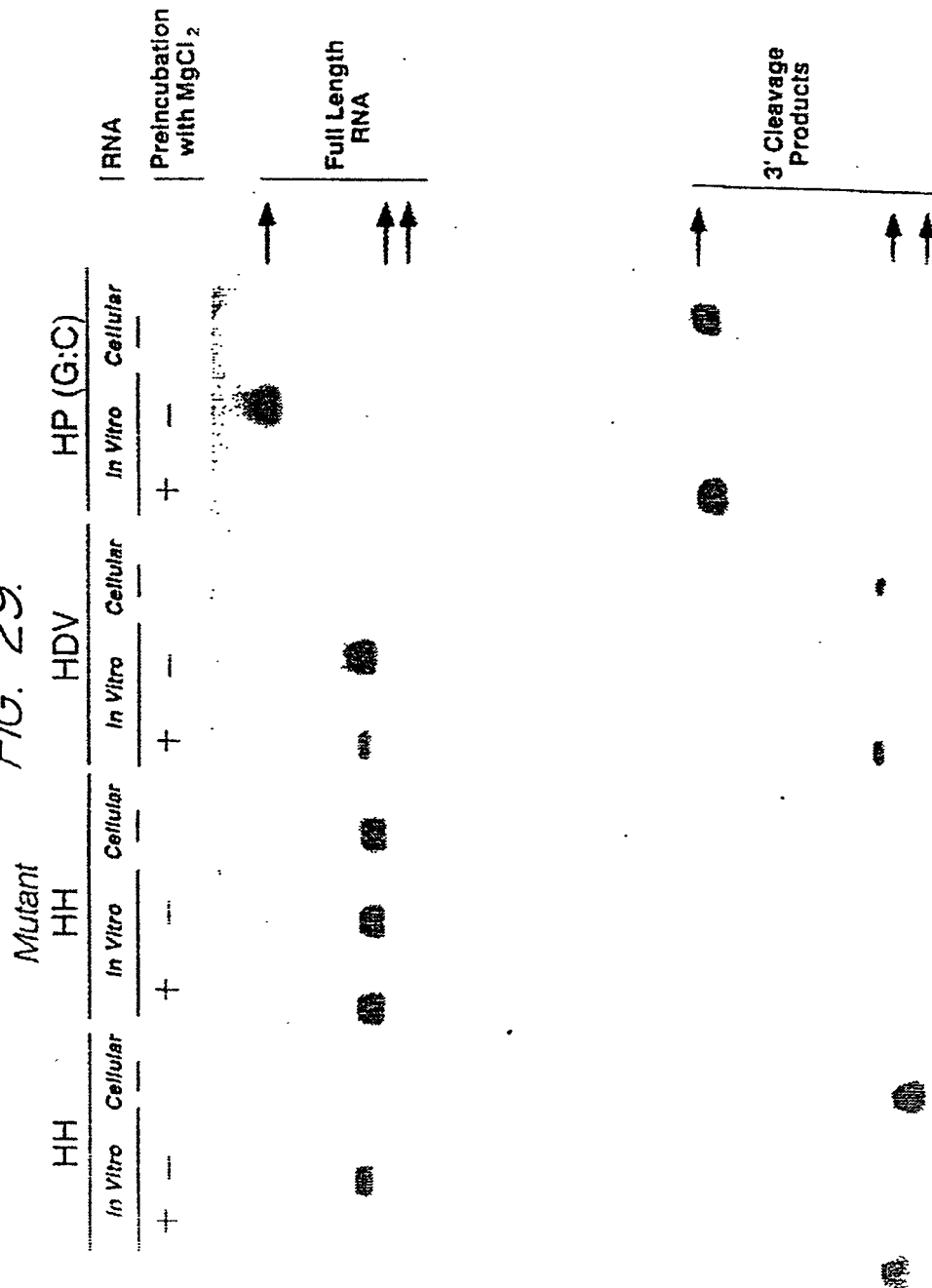
FIG. 28.

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FIG. 29.



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FIG. 30

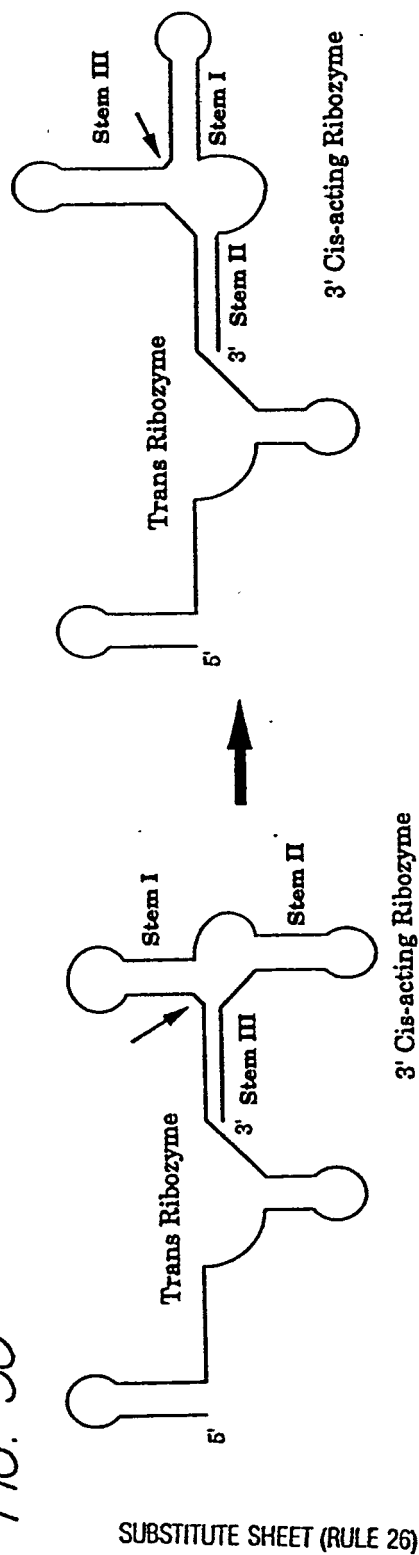
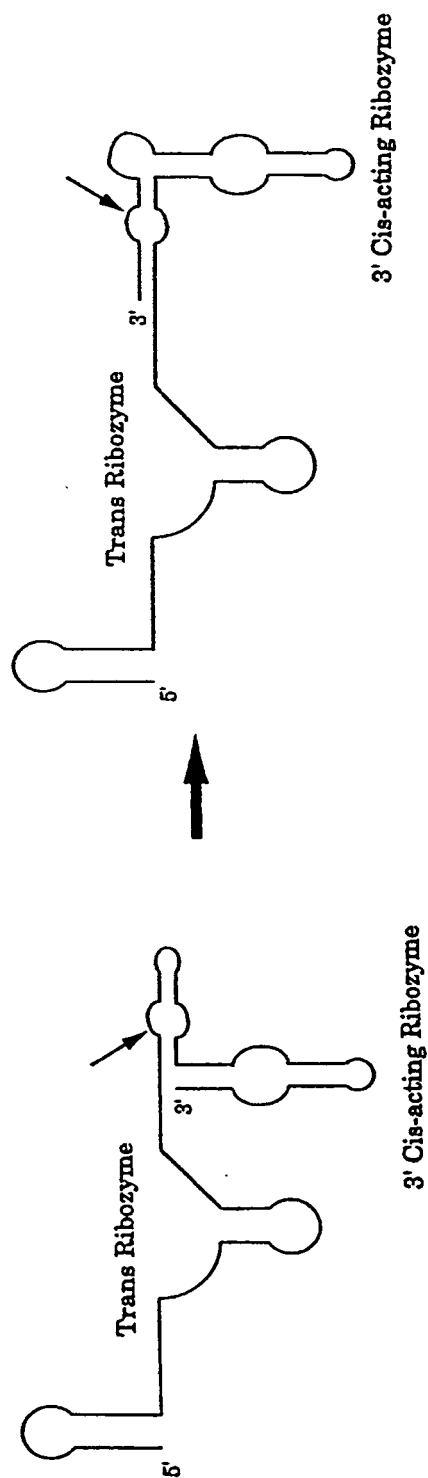


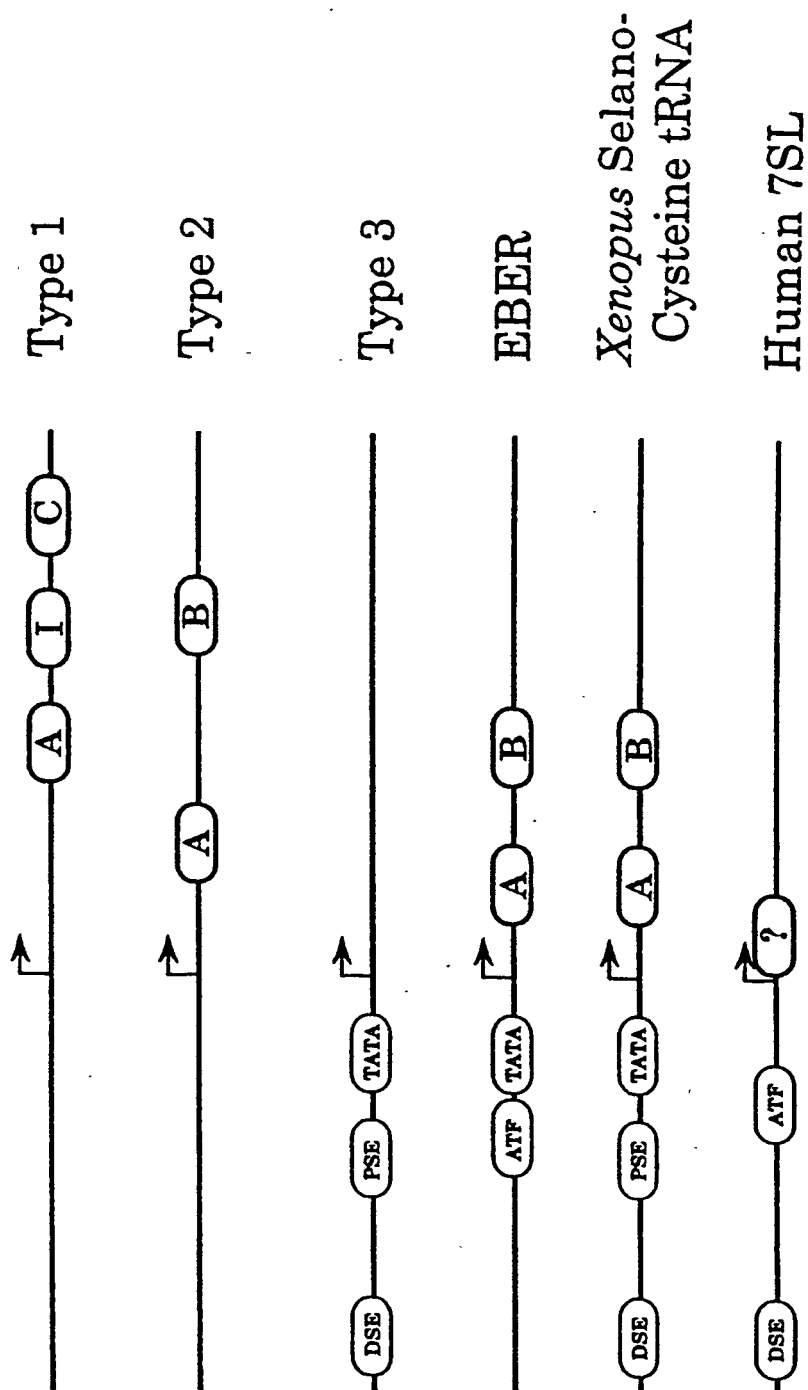
FIG. 31.



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FIG. 32.



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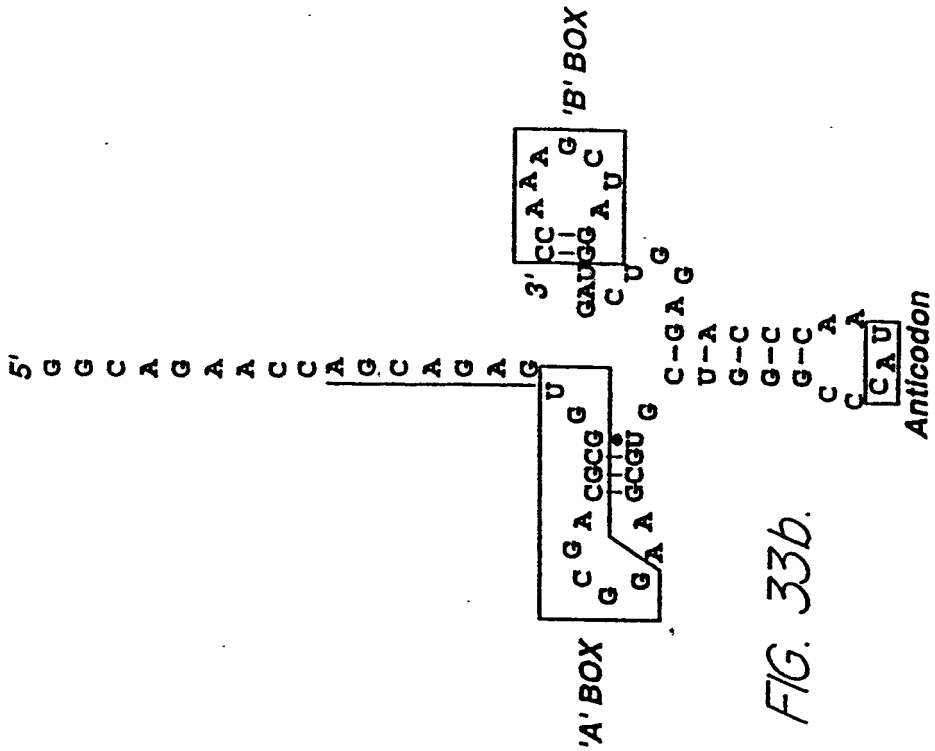


FIG. 33b.

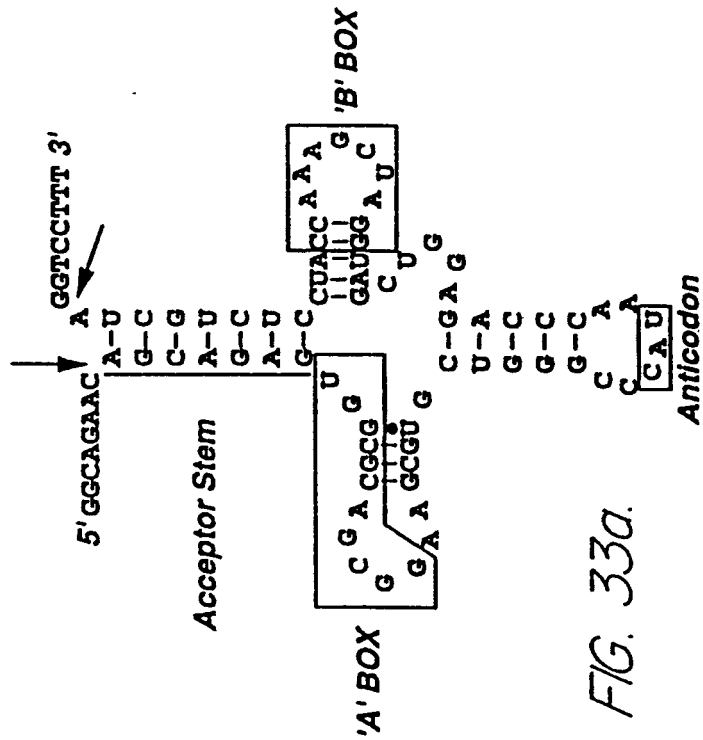


FIG. 33a.

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FIG. 34a.

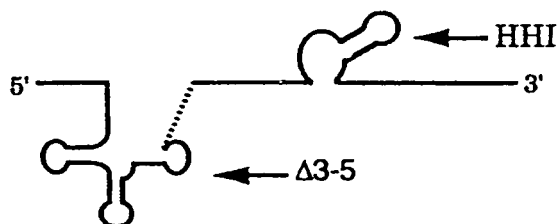
 $\Delta 3-5$ /HHI

FIG. 34b.

S3

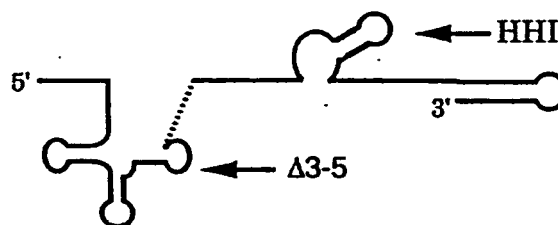


FIG. 34c.

S5

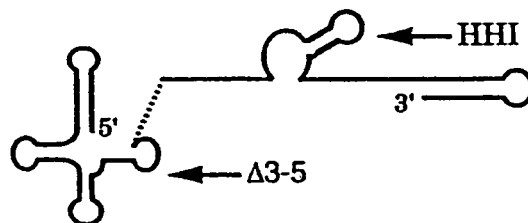


FIG. 34d.

S35

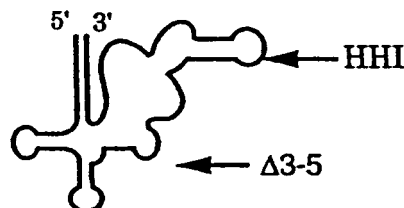
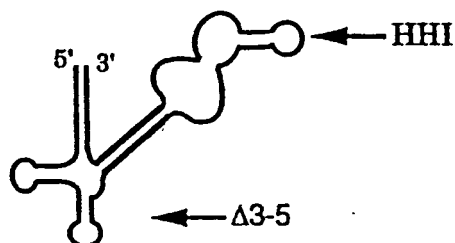


FIG. 34e.

S35Plus



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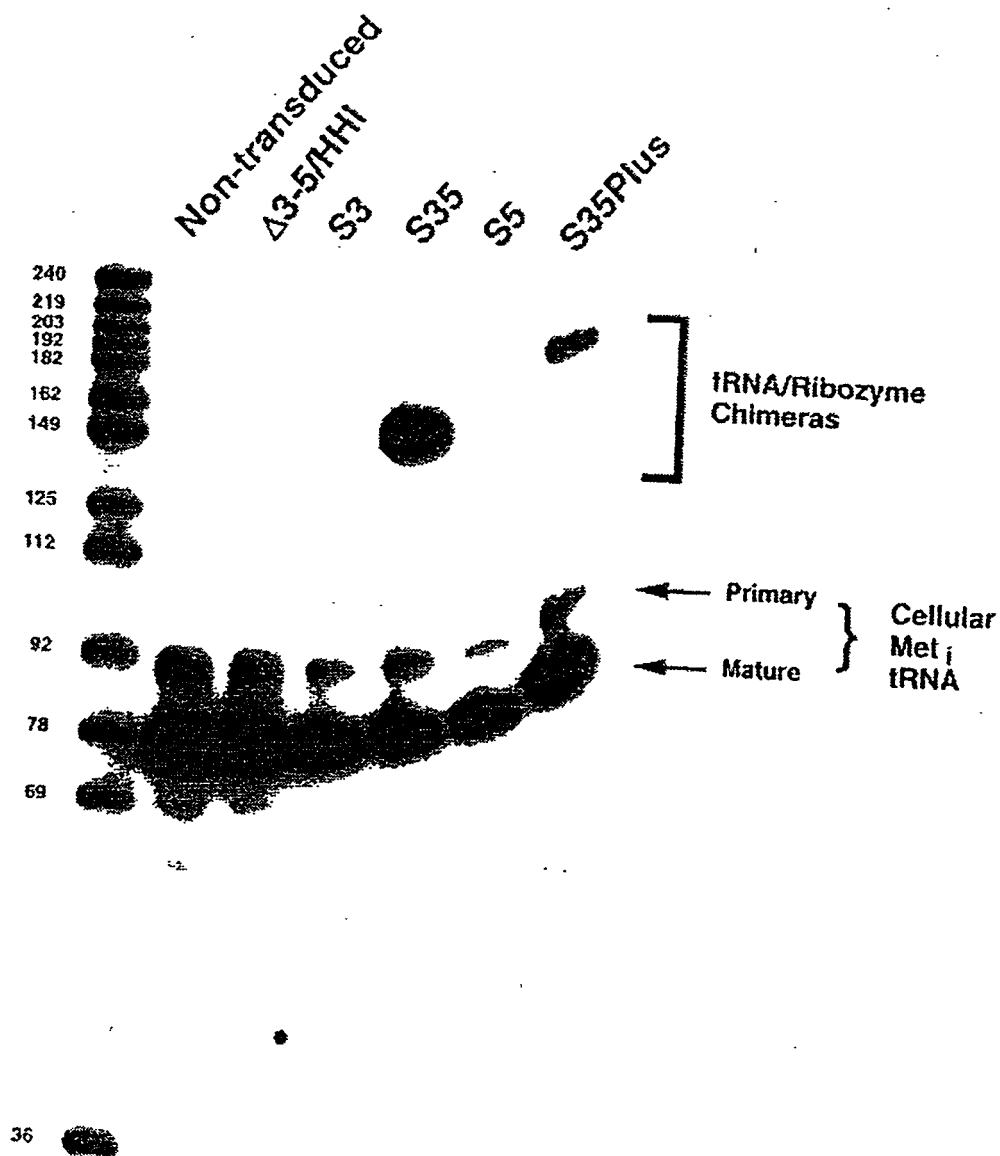


FIG. 35.

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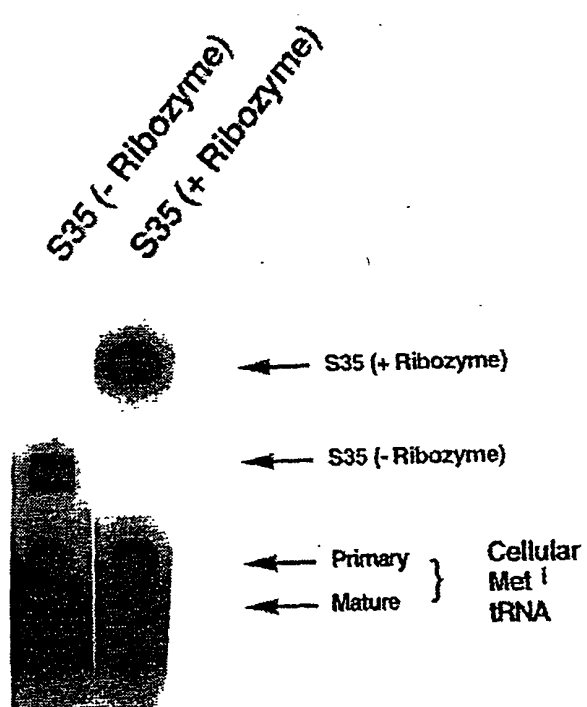


FIG. 36.

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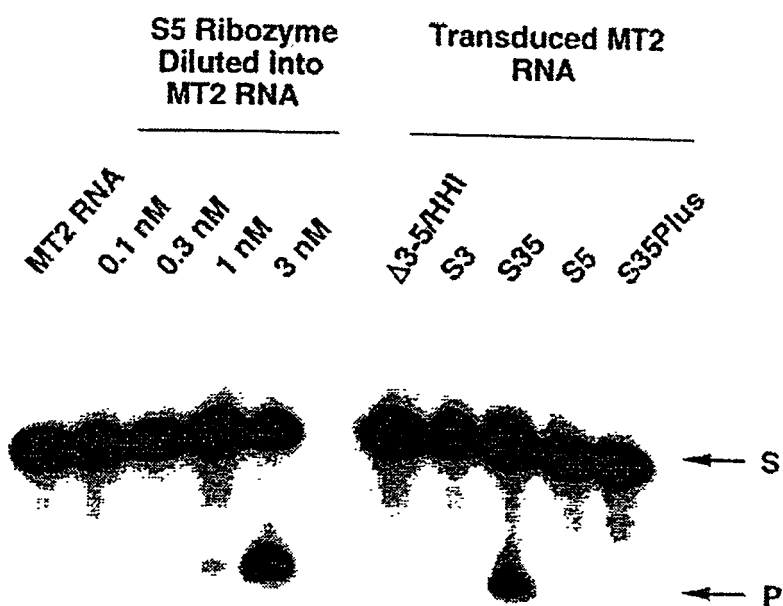
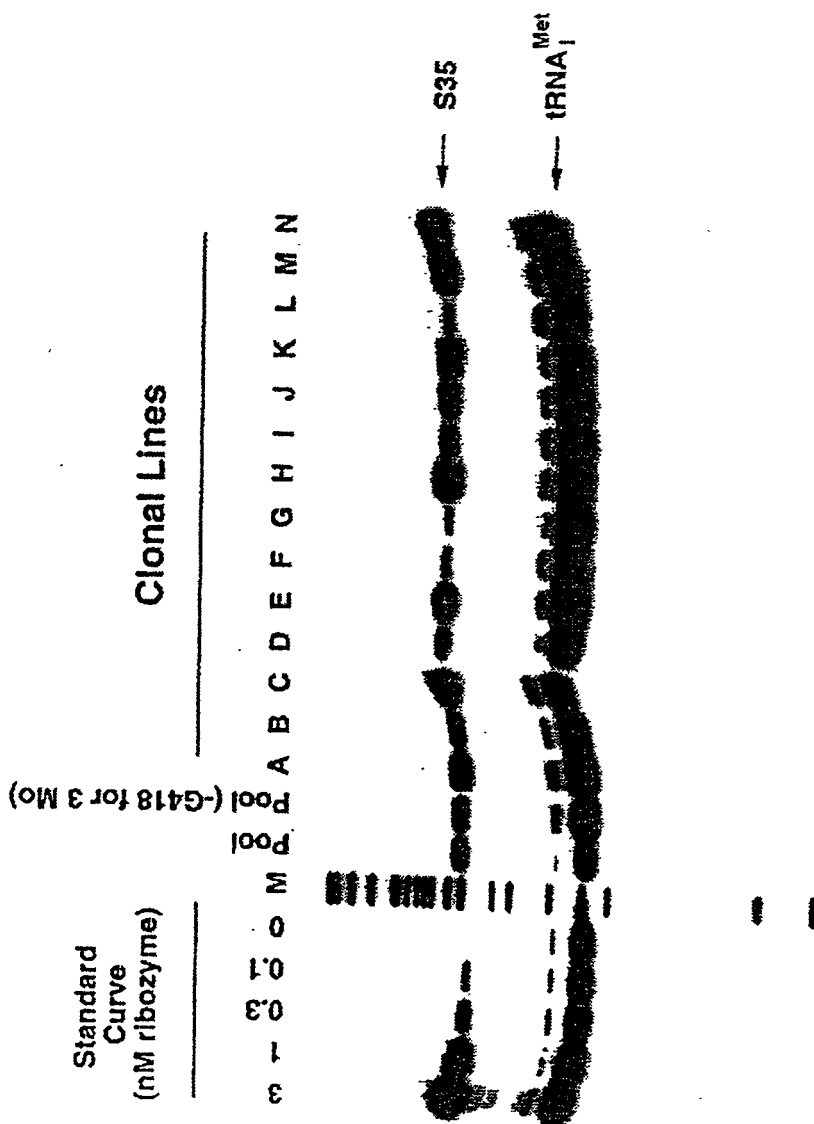


FIG. 37.

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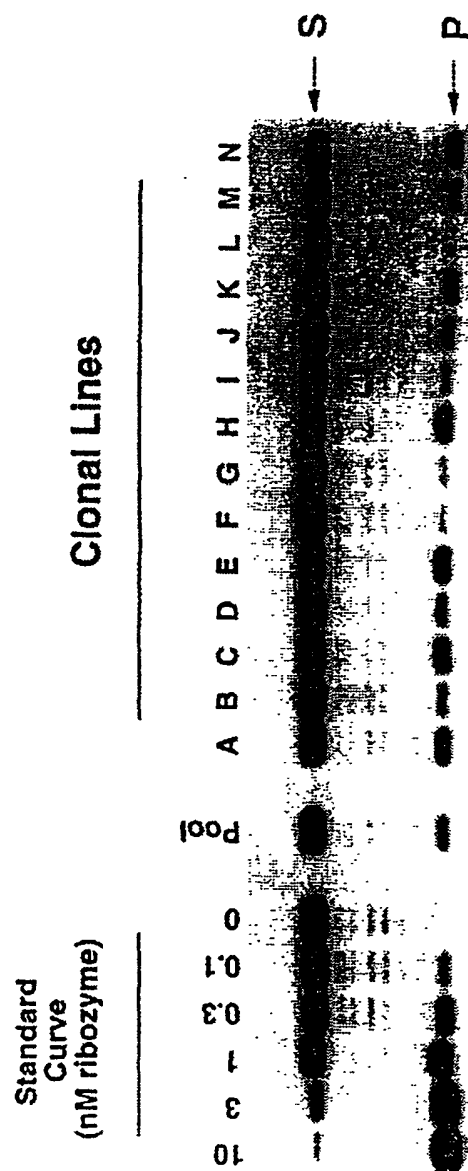
FIG. 38



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FIG. 39.



SUBSTITUTE SHEET (RULE 26)

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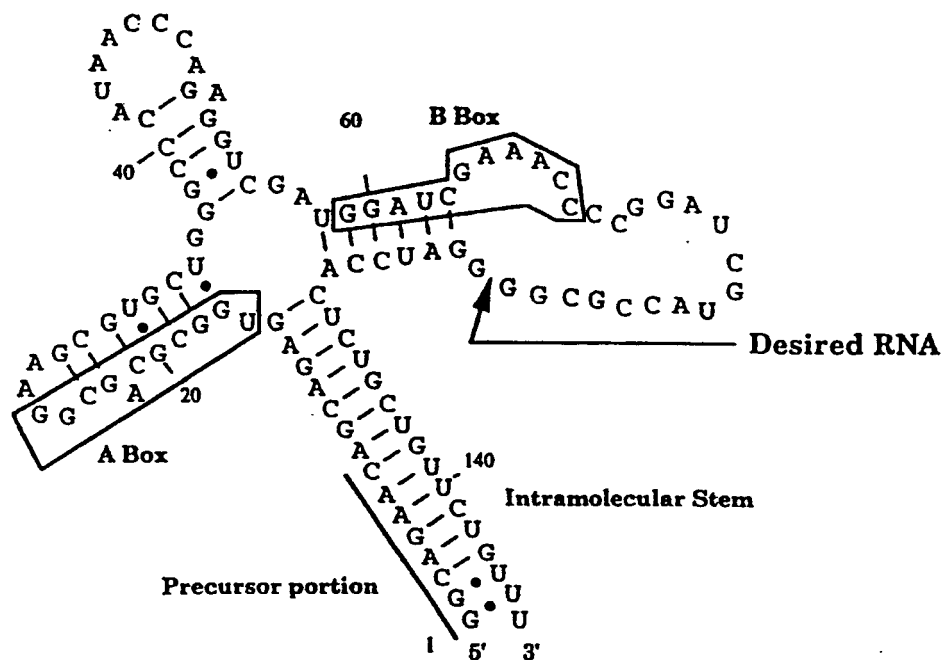


FIG. 40.

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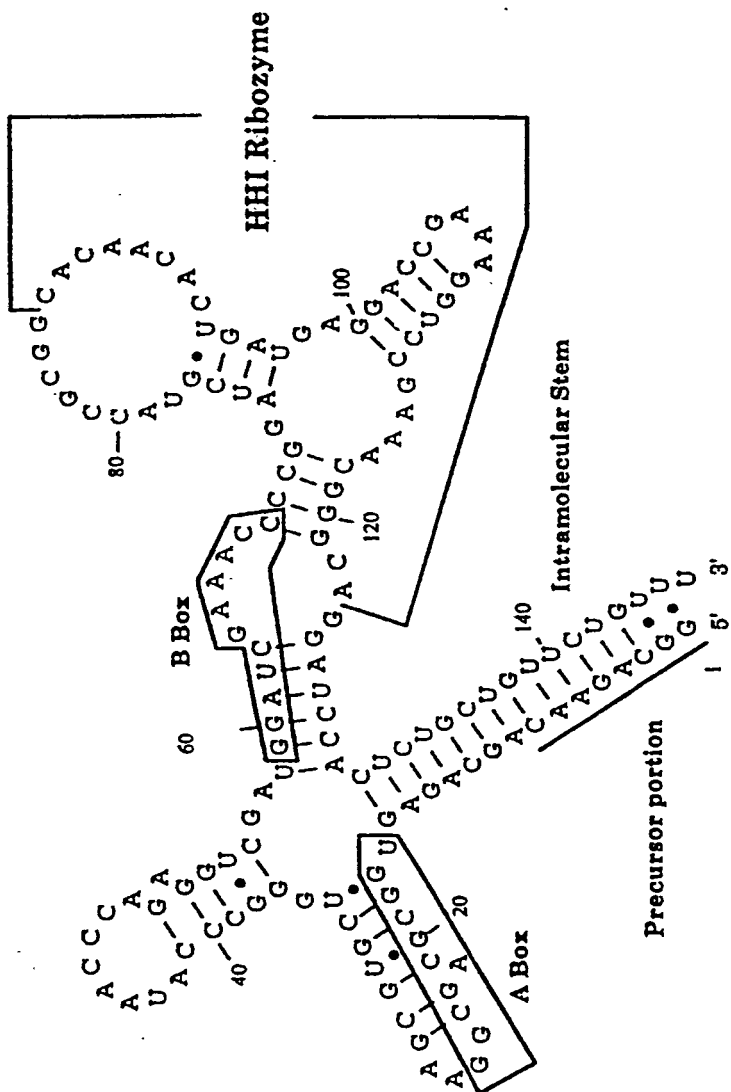
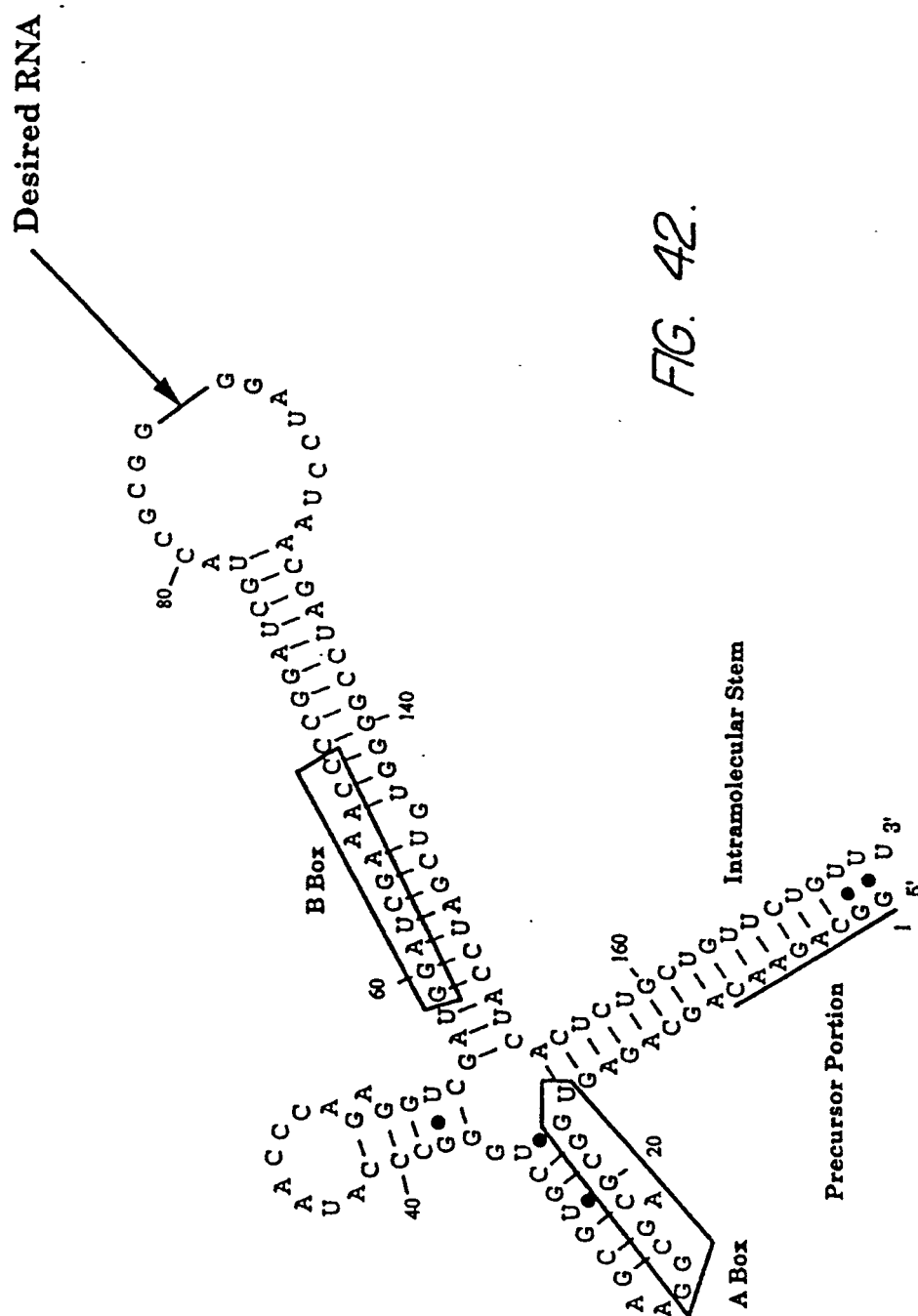


FIG. 41.



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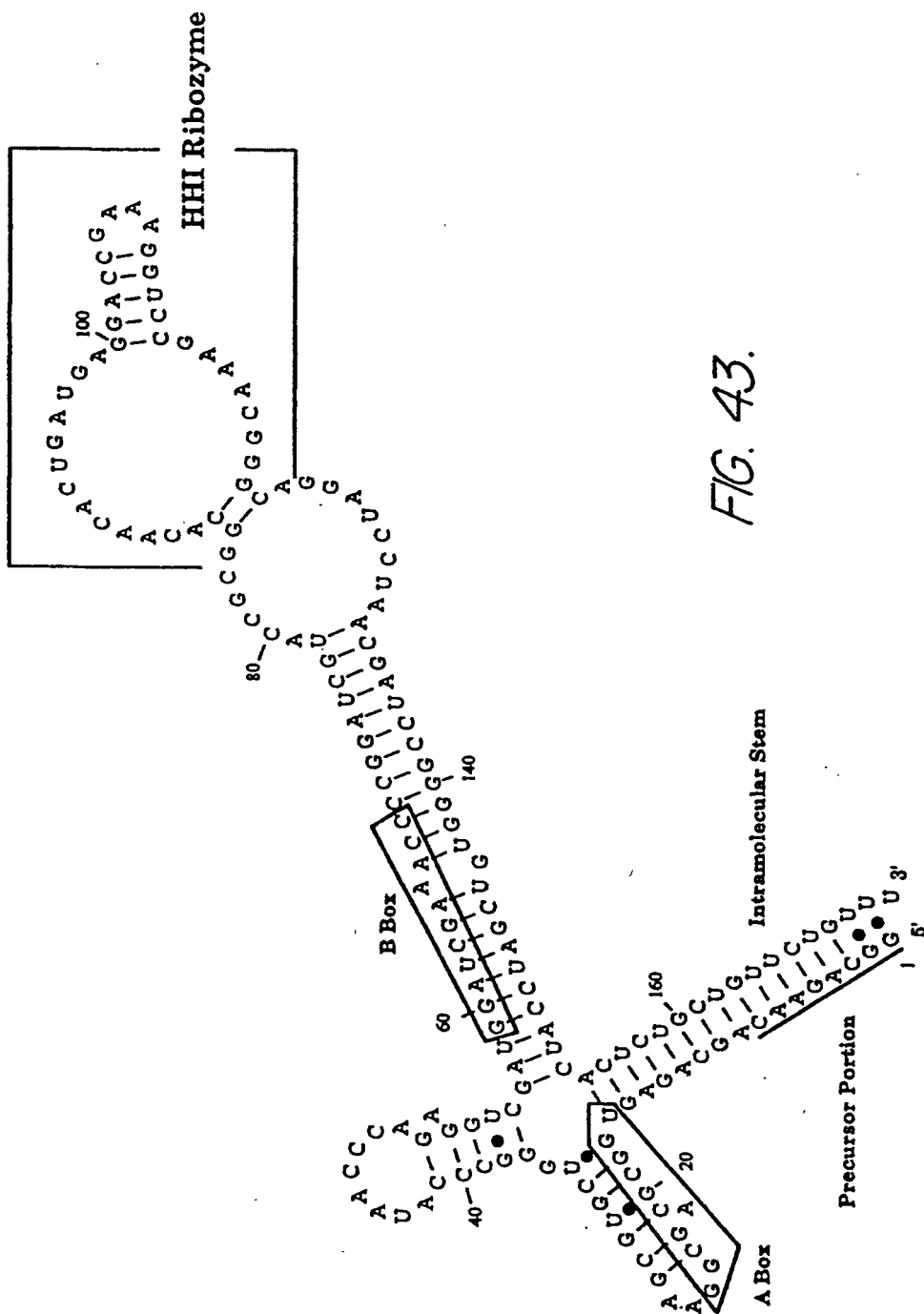


FIG. 43.

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*FIG. 44.***S35 Sequence**

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGUGGAU CCACUCUGCU 100
 GUUCUGUUU 109

*FIG. 45.***HHIS35**

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGCACAA CACUGAUGAG 100
GACCGAAAGG UCCGAAACGG GCAGGAUCCA CUCUGCUGUU CUGUUU 146

Underlined bases indicate the HHI ribozyme sequence

*FIG. 46.***S35 Plus Sequence**

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGGGAUC CUAACGAUCC 100
 GGGGUGUCGA UCCAUCACUC UGCUGUUCUG UU U 133

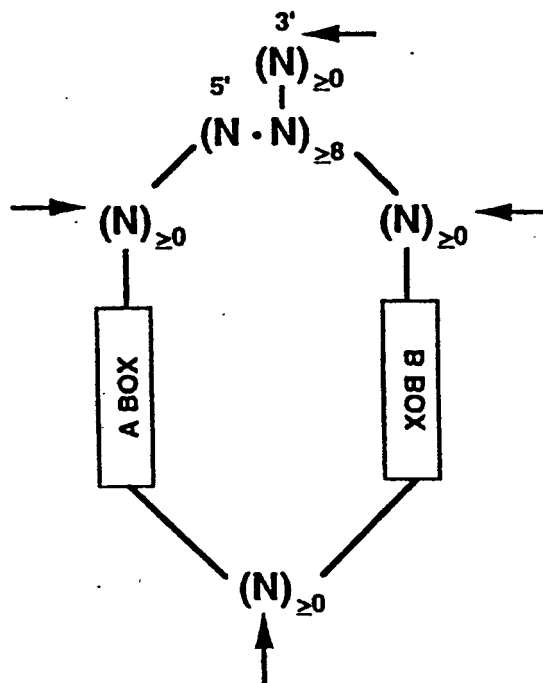
*FIG. 47.***HHIS35 Plus**

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGCACAA CACUGAUGAG 100
GACCGAAAGG UCCGAAACGG GCAGGAUCCU AACGAUCCGG GGUGUCGAUC 150
 CAUCACUCUG CUGUUCUGUU U 171

Underlined bases indicate the HHI ribozyme sequence
 SUBSTITUTE SHEET (RULE 26)

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FIG. 48.



A BOX = URGCNNAGYGG

B BOX = GGUUCGANUCC

This is based on Geiduschek & Tocchini-Valentini, (1988) Annu. Review Biochem. 57, 873-914. However this consensus sequence is not meant to be limiting

N = A, U, G, or C

R = Purine

Y = Pyrimidine

• = Indicates base-pairing

— = Indicates covalent linkage

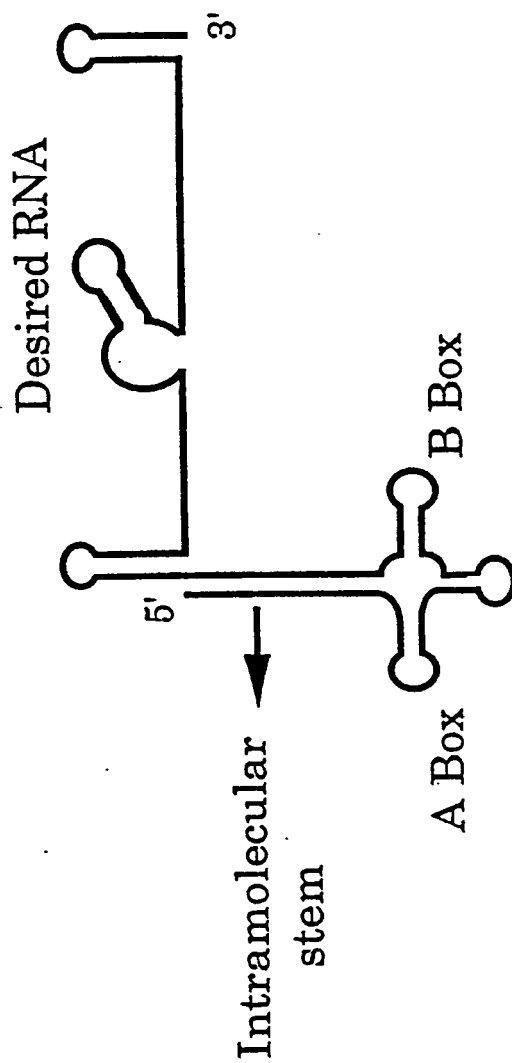
➡ = Indicates sites at which desired RNAs can be cloned

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FIG. 49.



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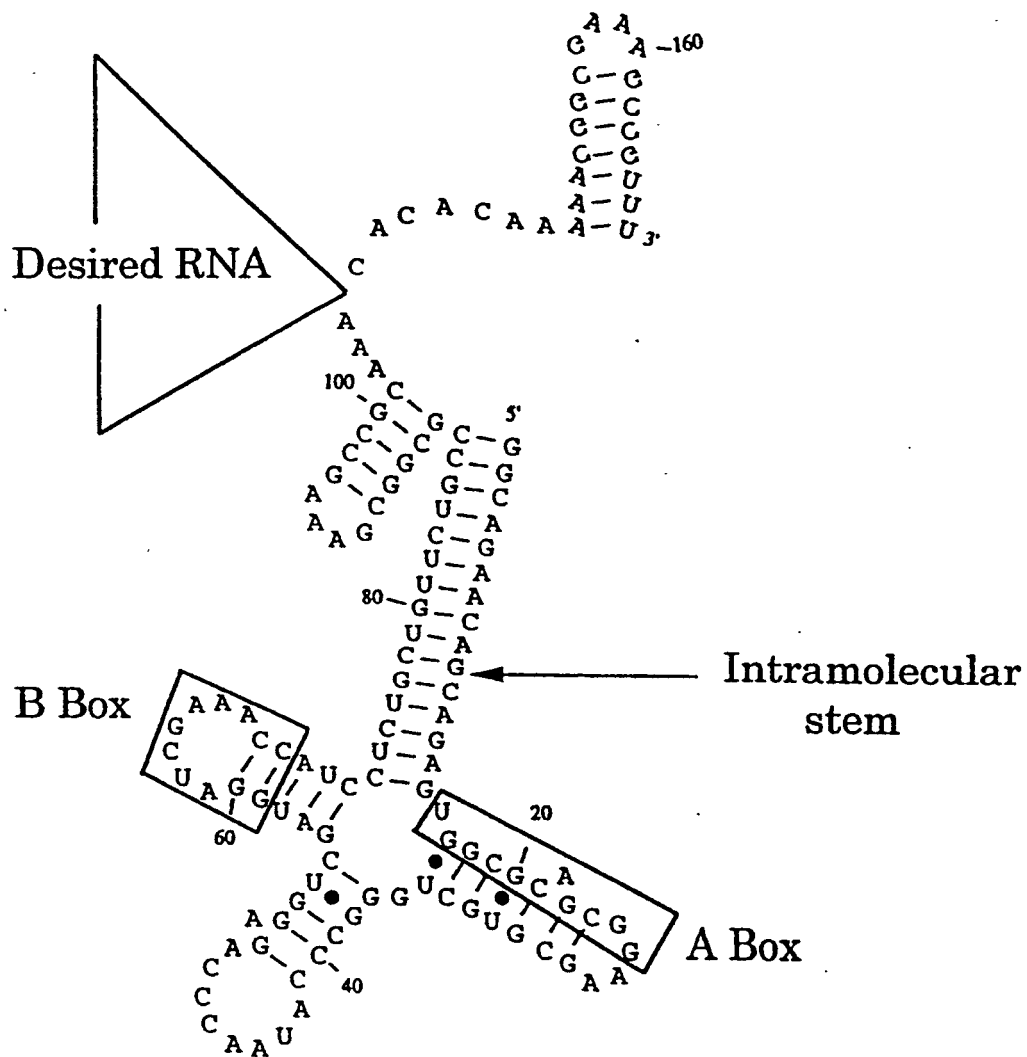


FIG. 50.

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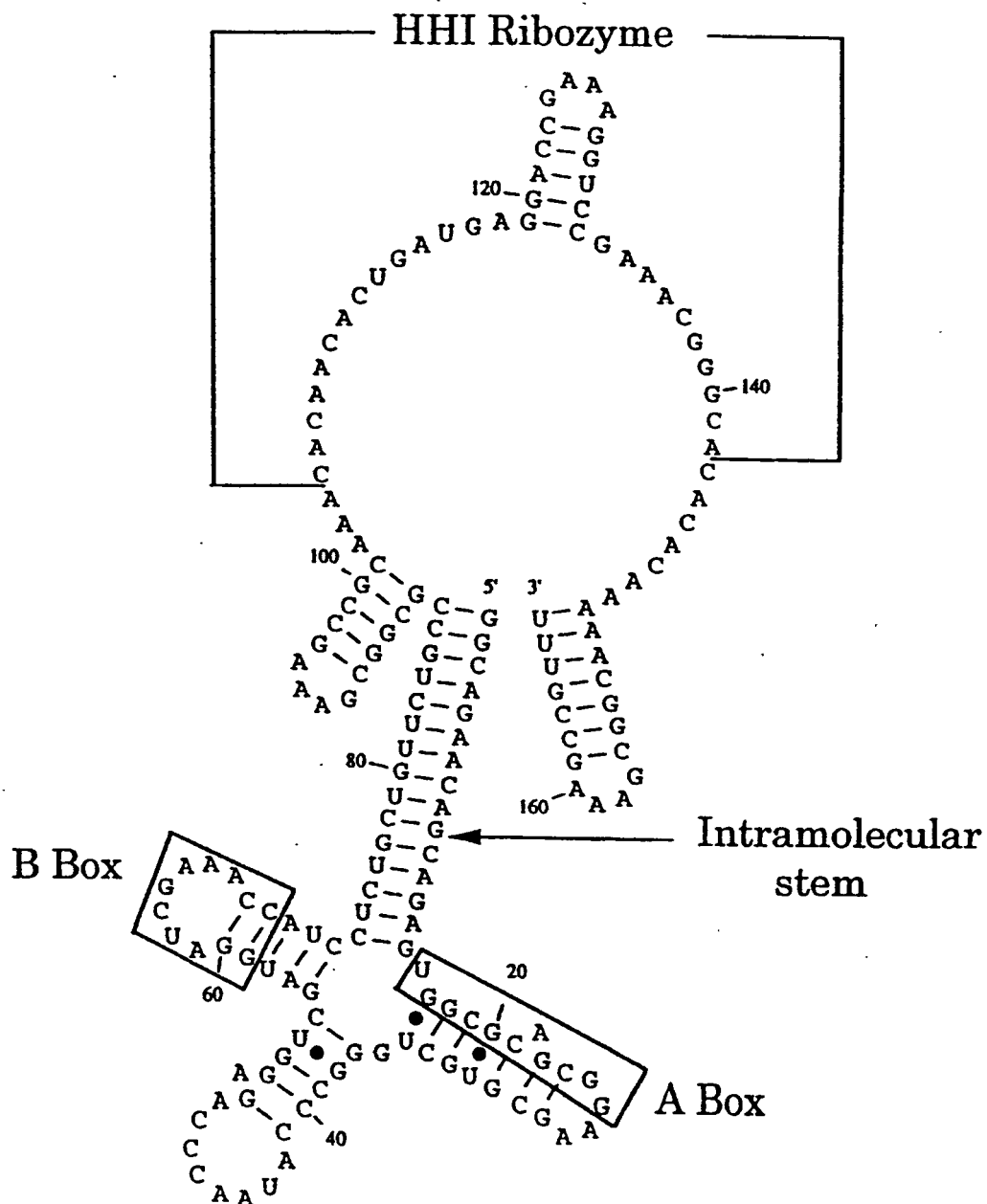


FIG. 51.

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FIG. 52a.

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A: TRZ-A

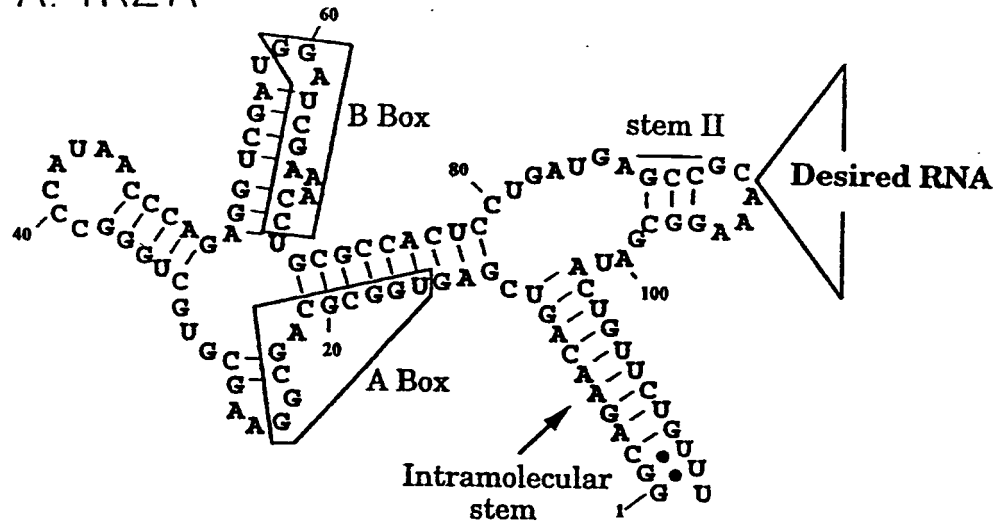
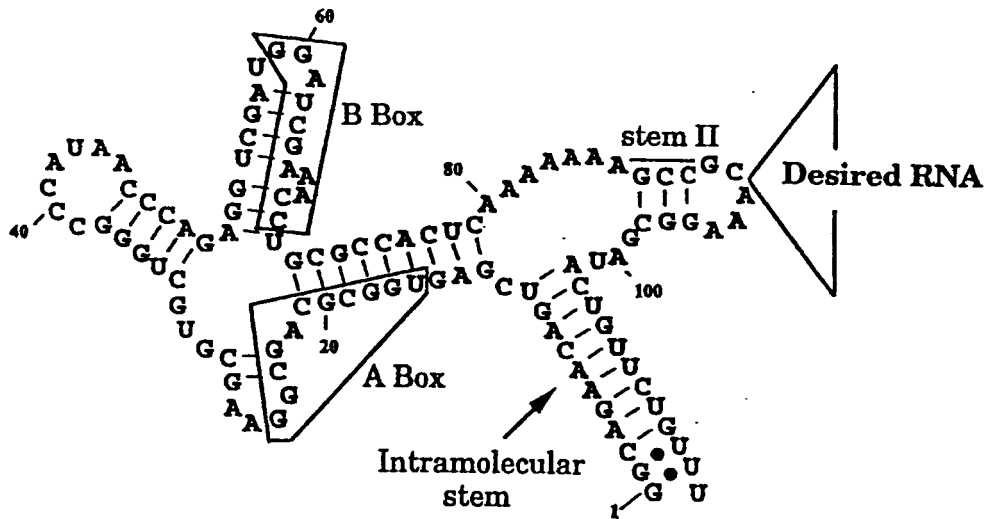
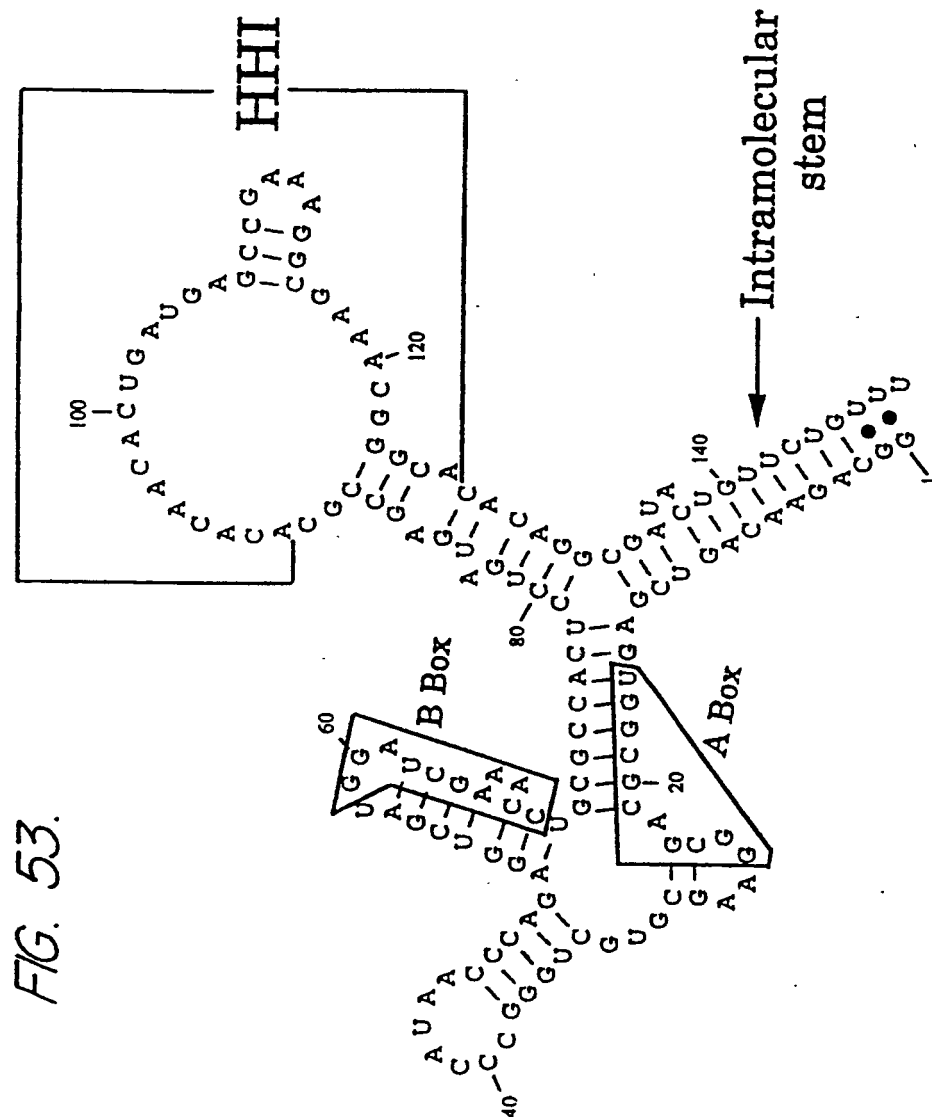


FIG. 52b.

B: TRZ-B



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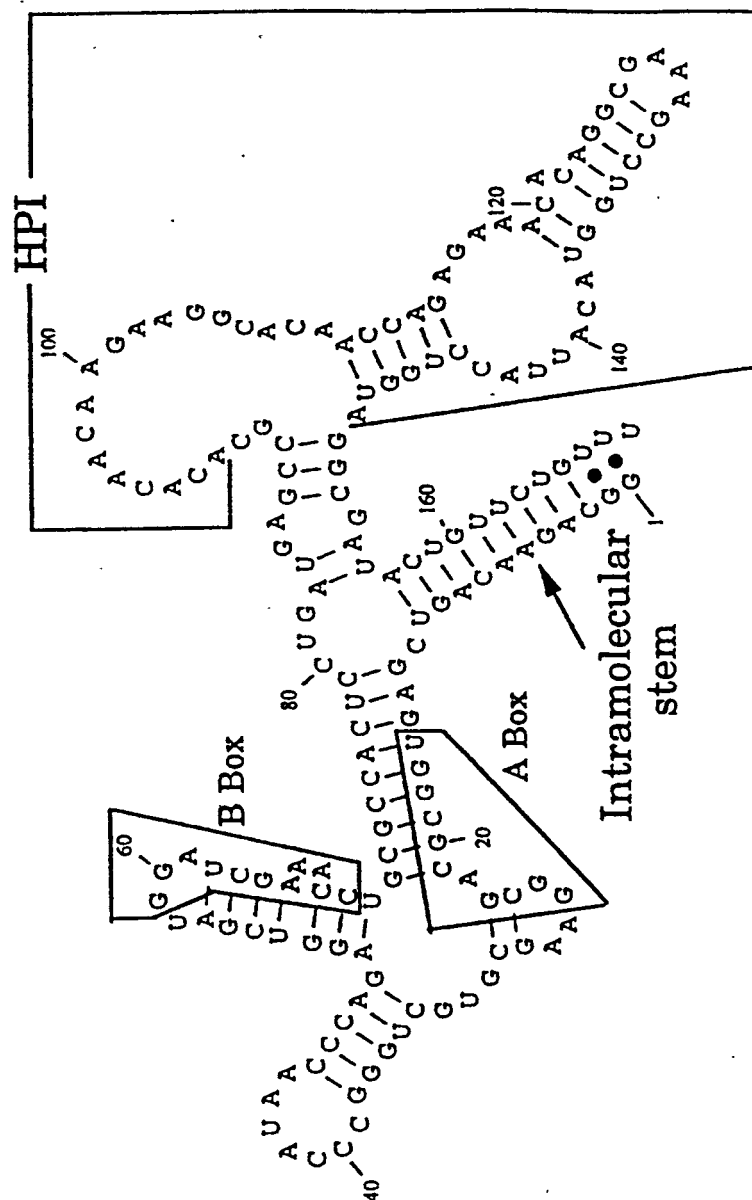


FIG. 54.

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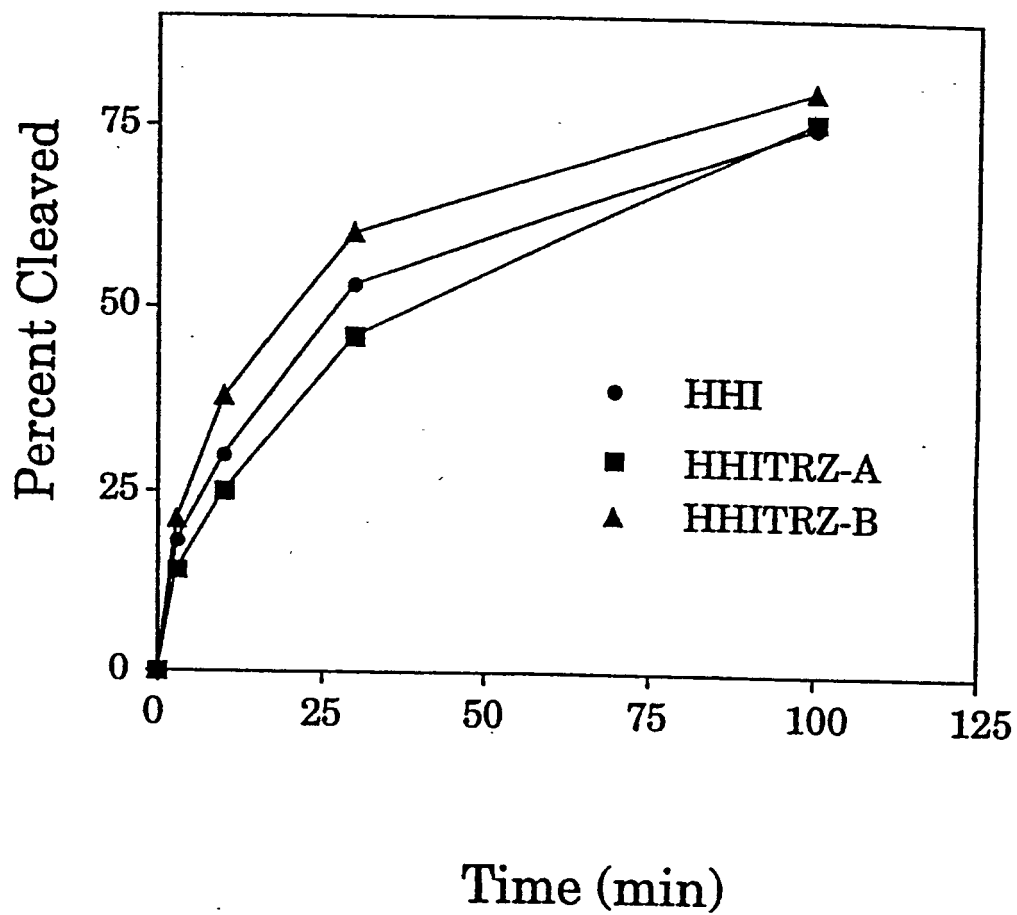


FIG. 55.

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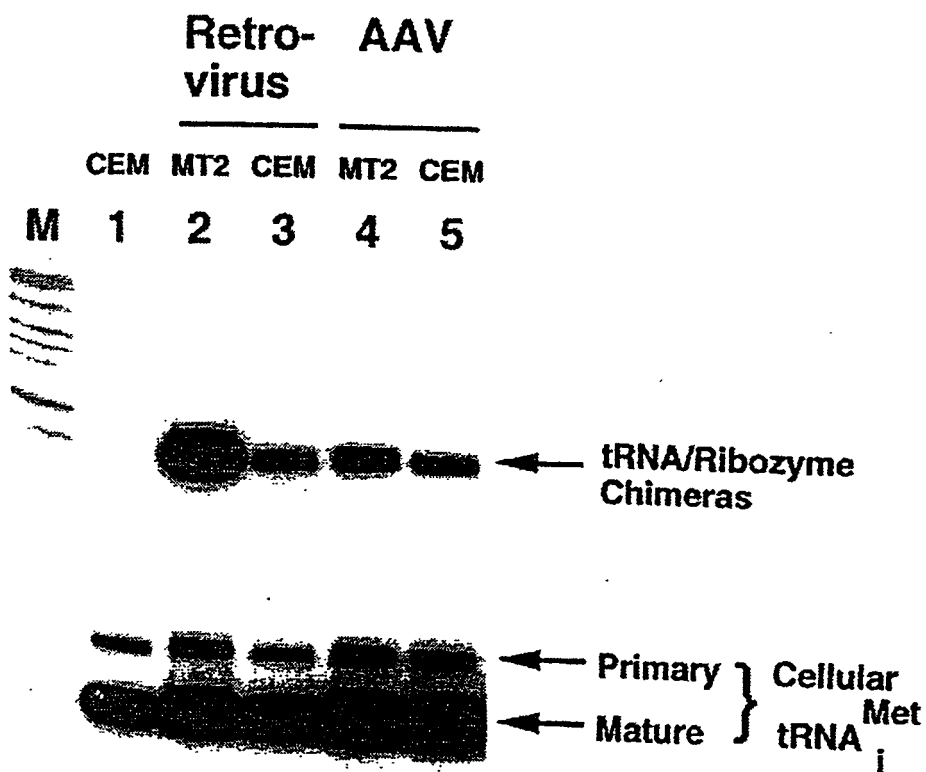


FIG. 56.

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FIG. 57a.

AAV Vector

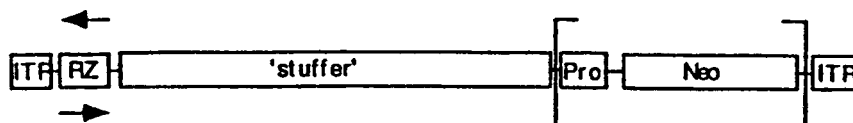
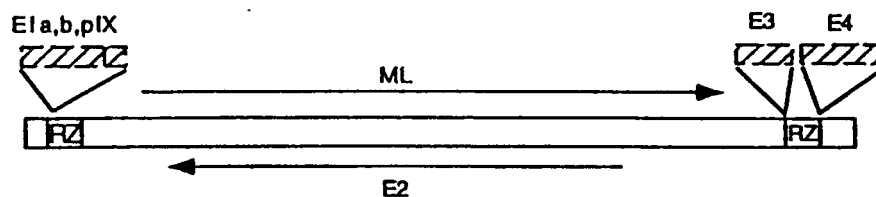


FIG. 57b.

Adenovirus Vector



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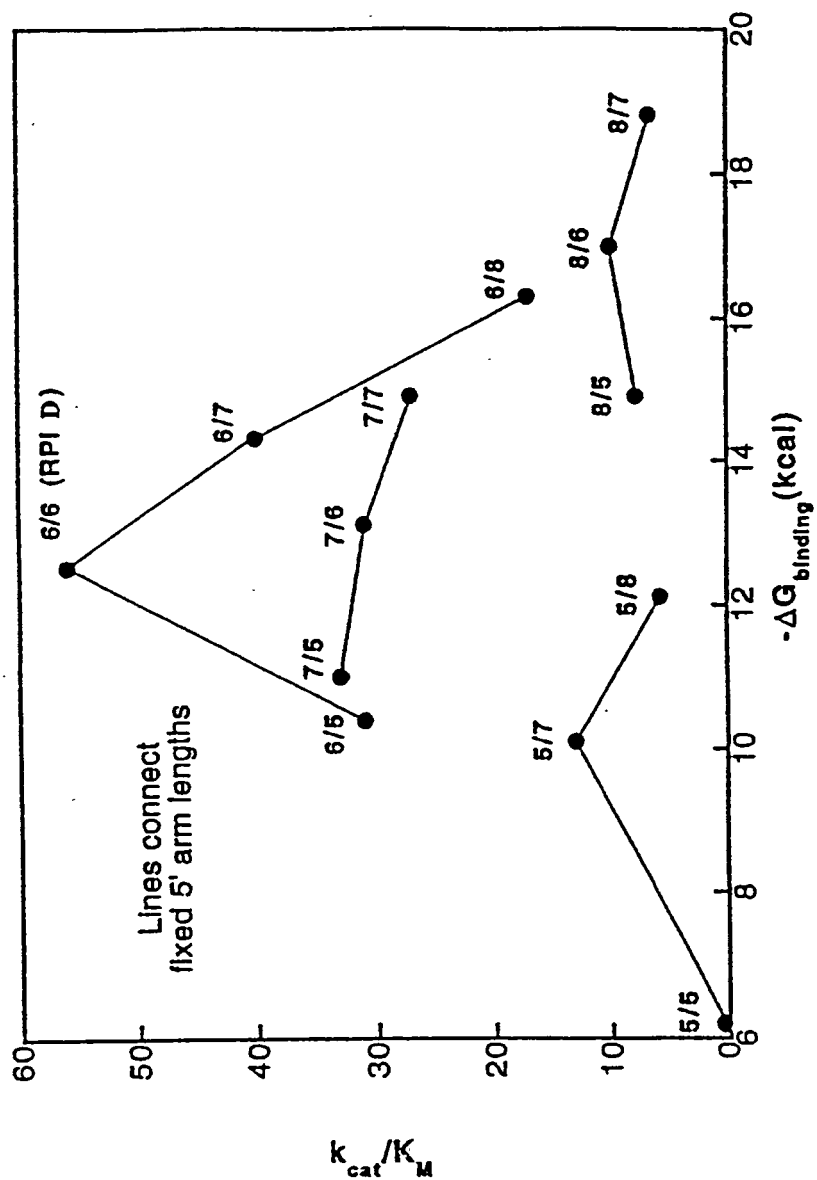
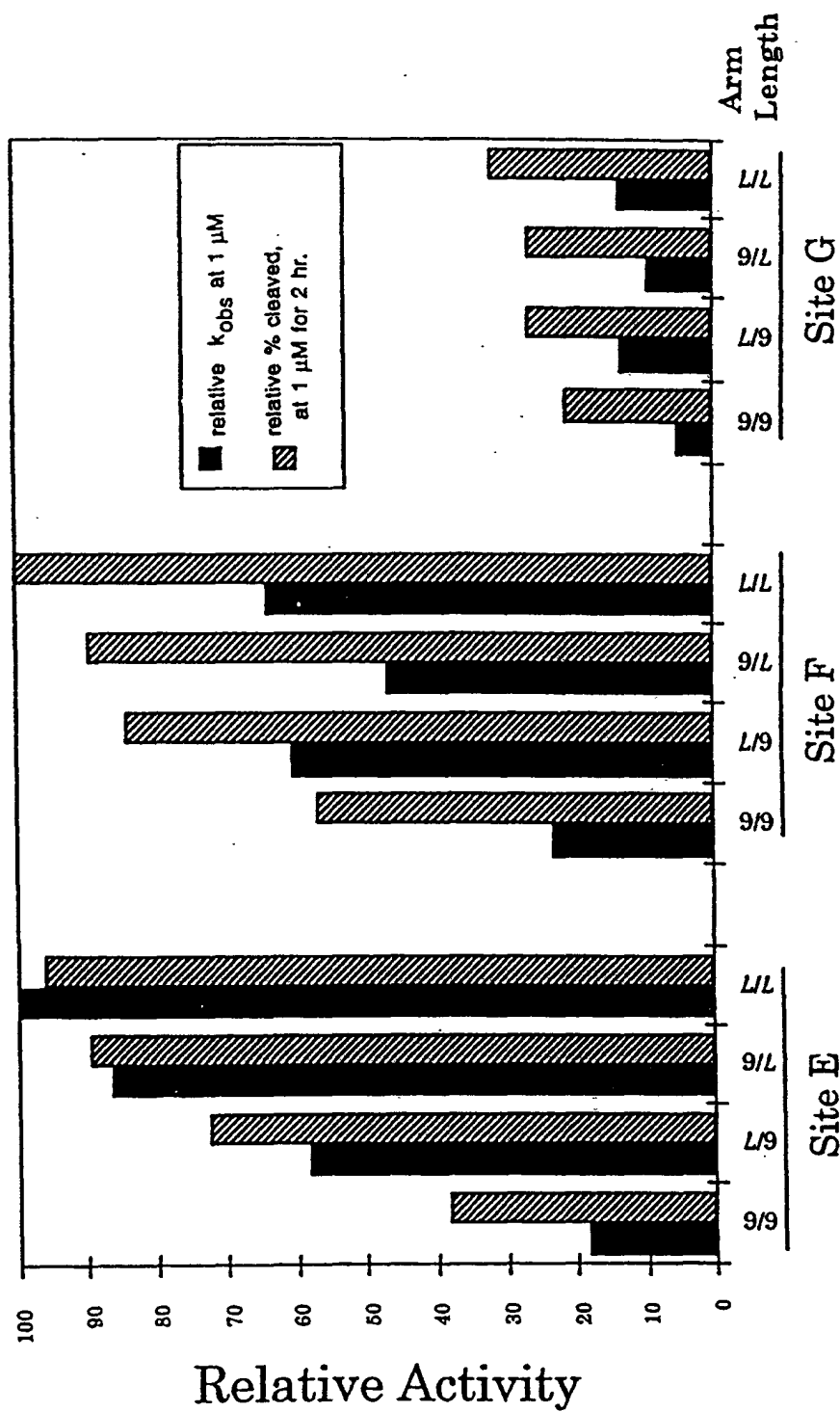


FIG. 58.

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Ribozyme

FIG. 59.

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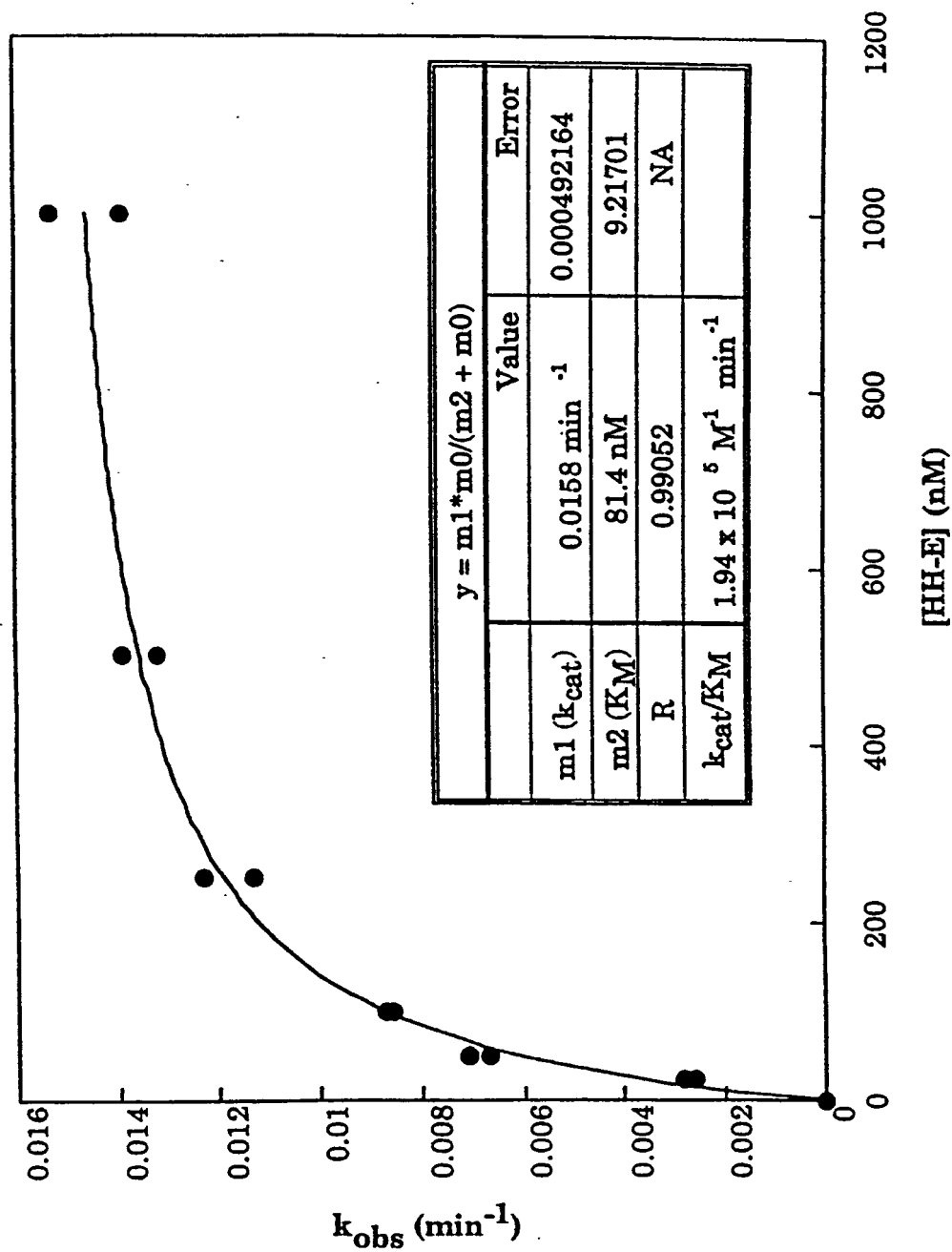
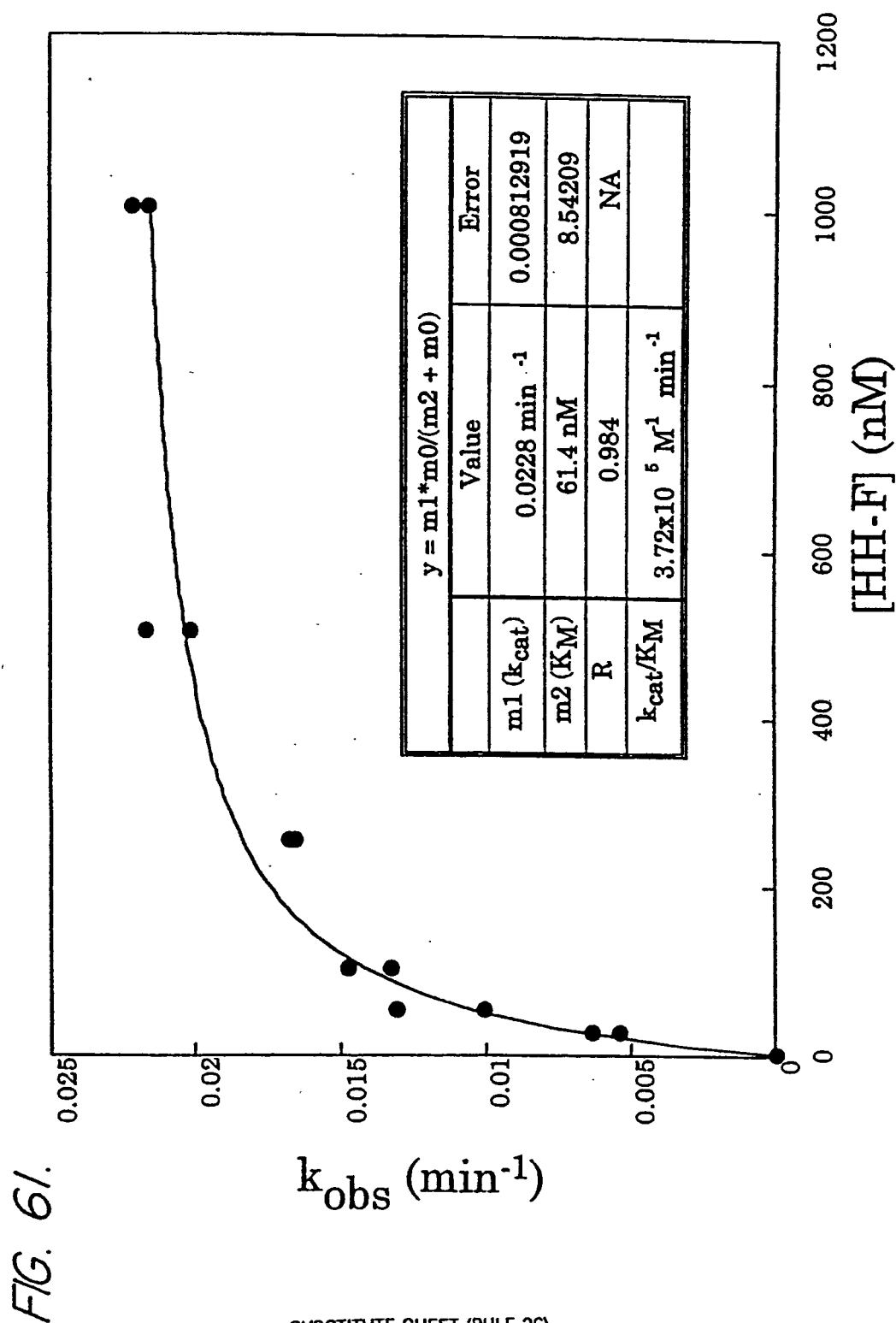


FIG. 60.

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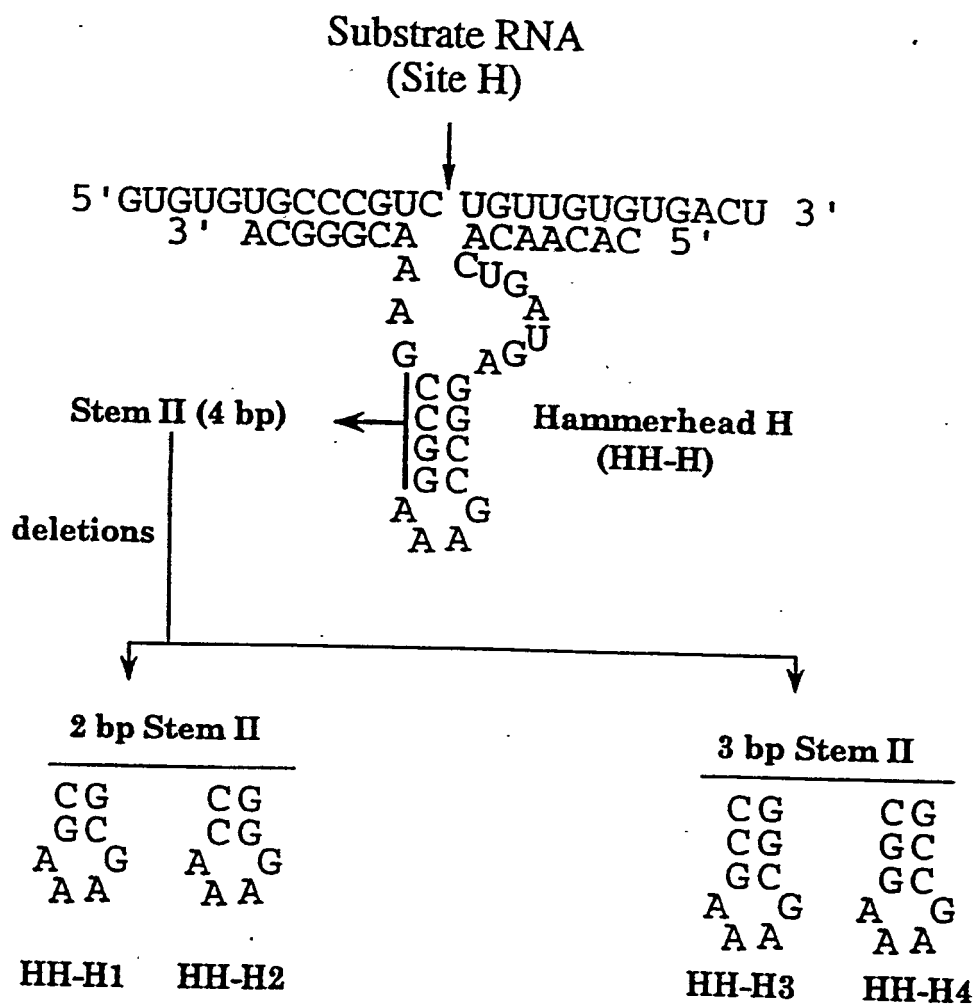


FIG. 62.

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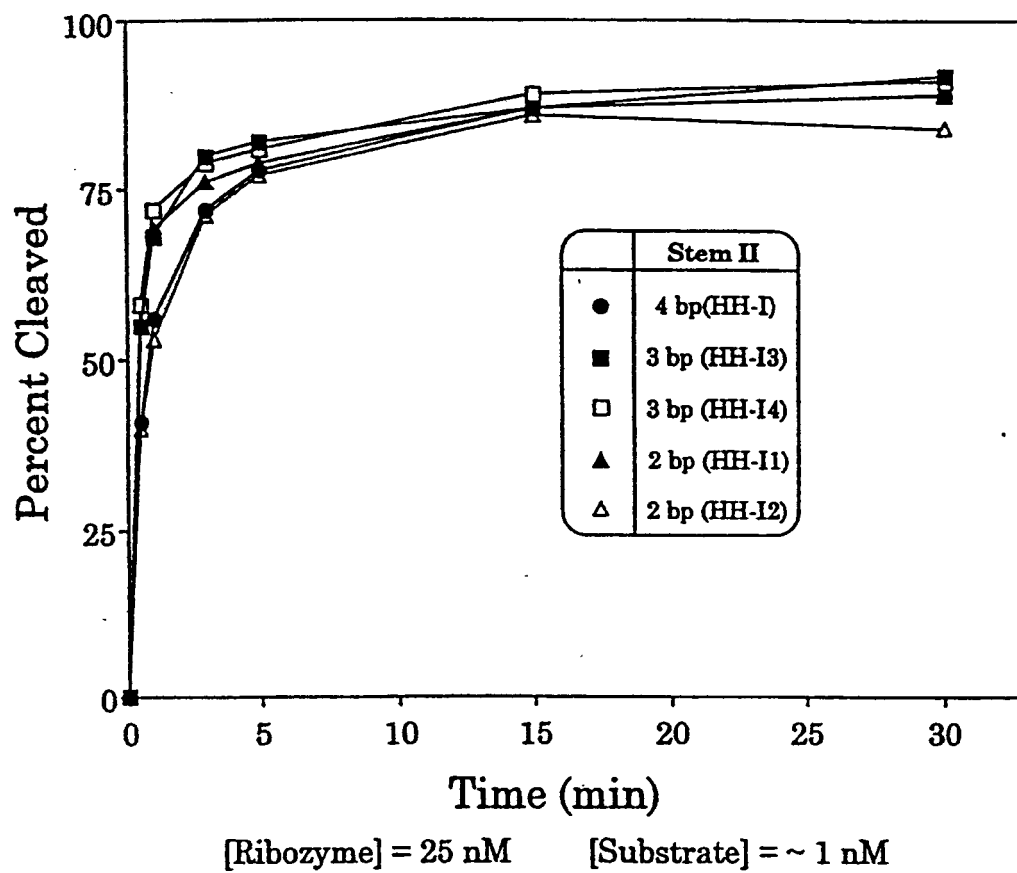
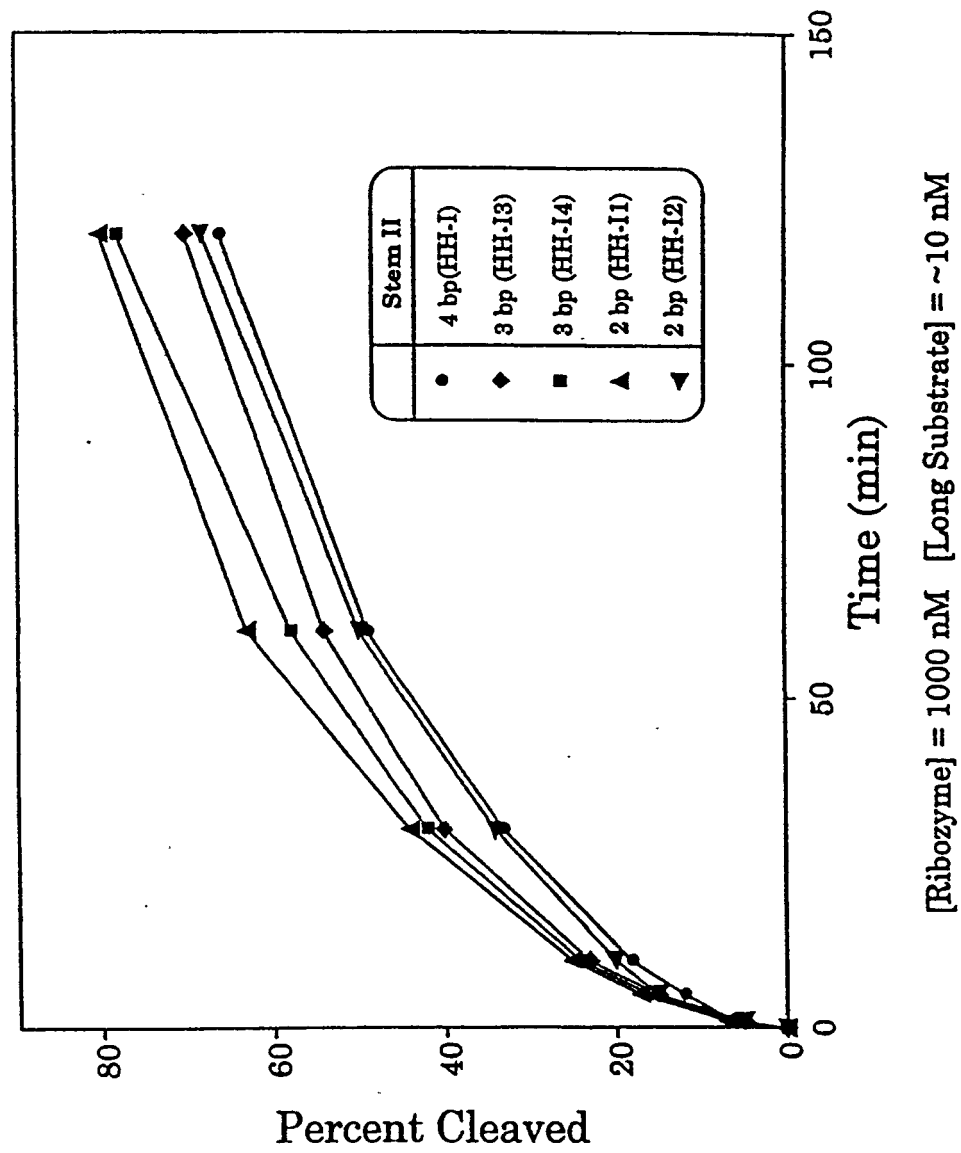


FIG. 63.

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[Ribozyme] = 1000 nM [Long Substrate] = ~10 nM

FIG. 64.

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FIG. 65a.

Substrate RNA (site J)

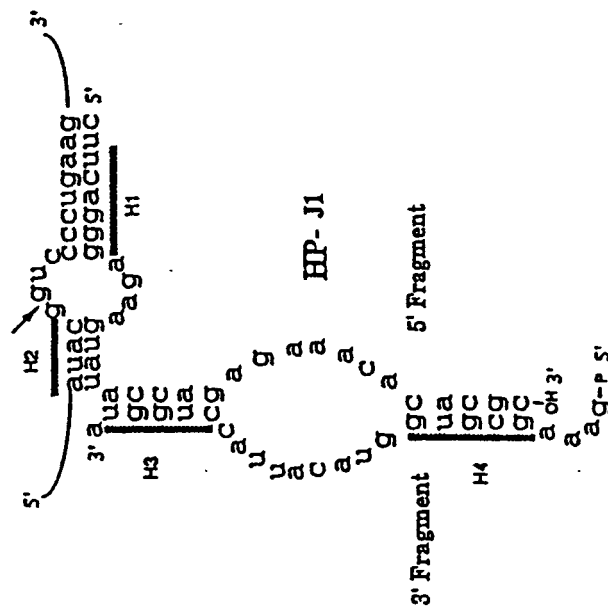
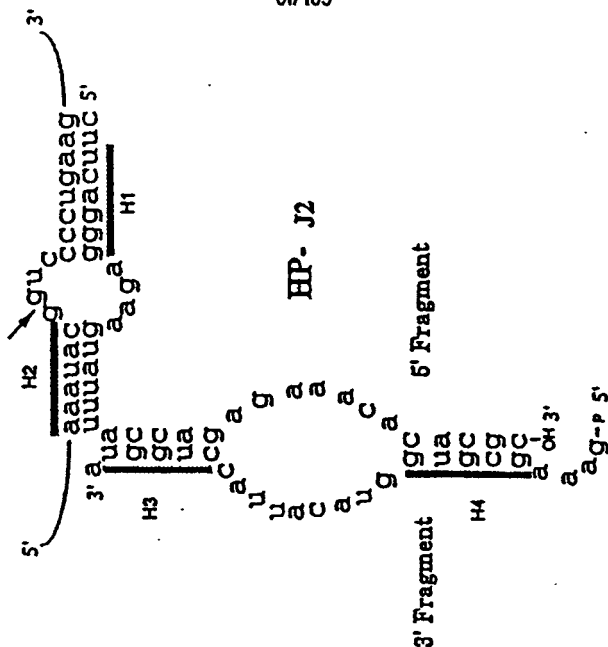


FIG. 65b.

Substrate RNA (site J)



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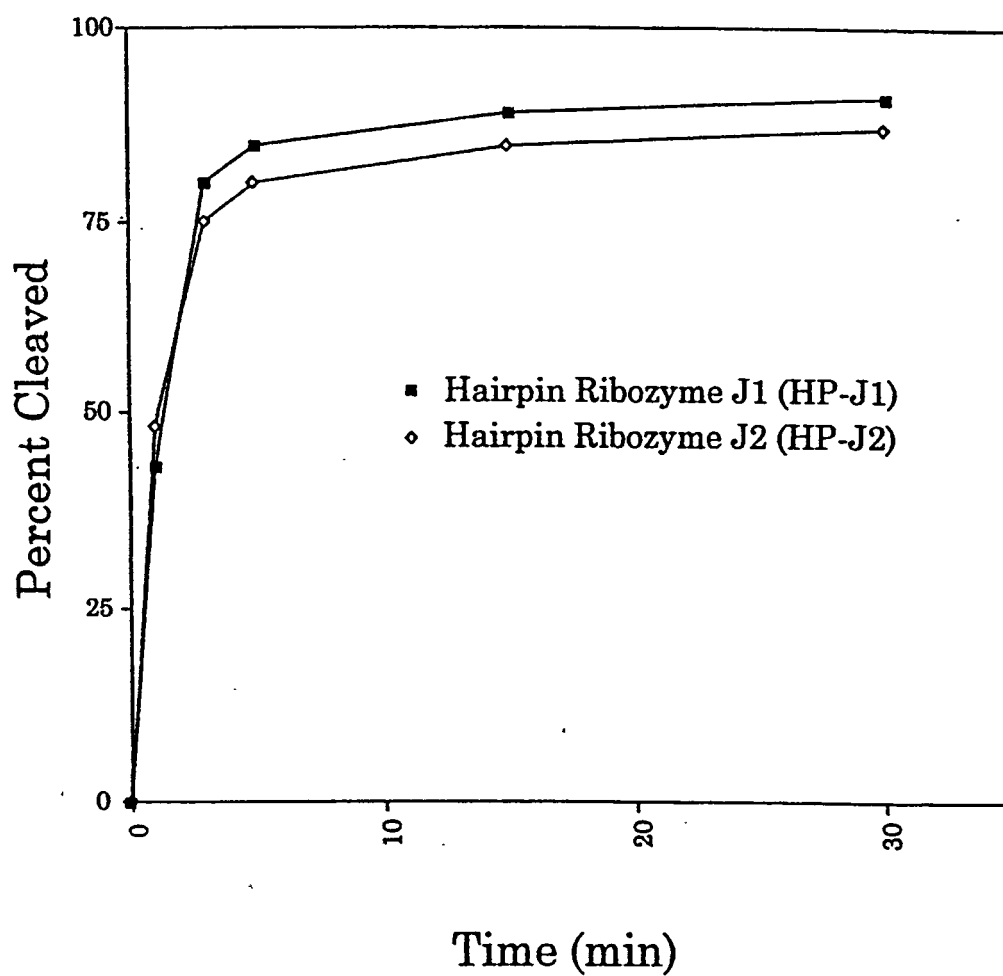


FIG. 66.

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FIG. 67b.

Substrate RNA

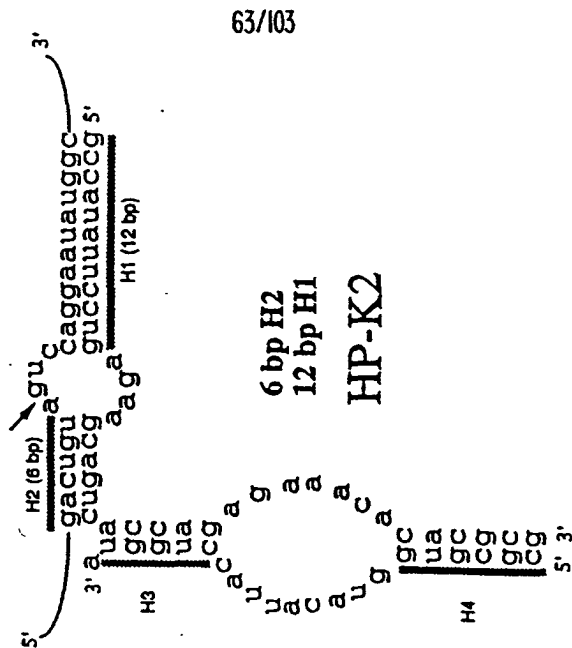
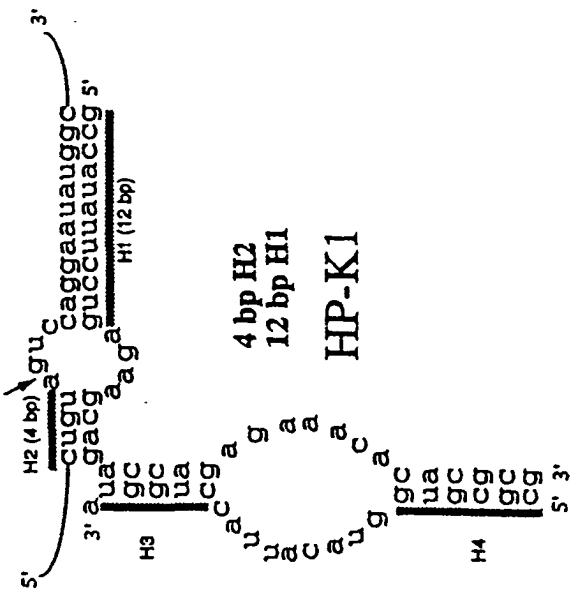
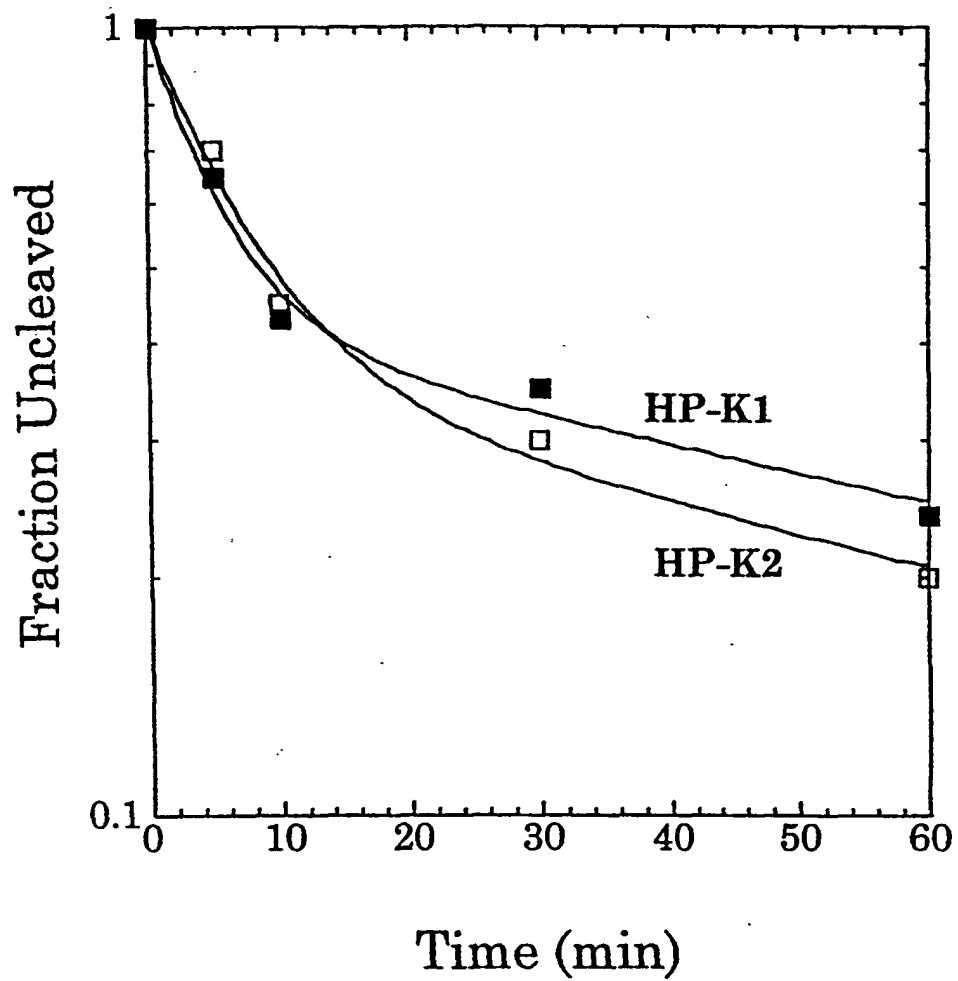


FIG. 67a.

Substrate RNA



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*FIG. 68.*

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FIG. 69b.

Substrate RNA

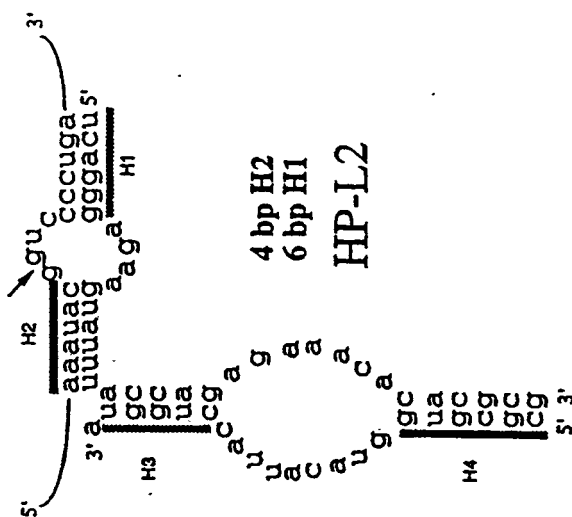
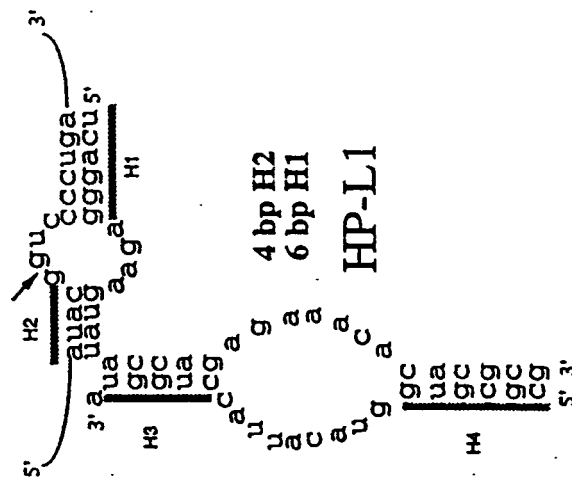


FIG. 69a.

Substrate RNA



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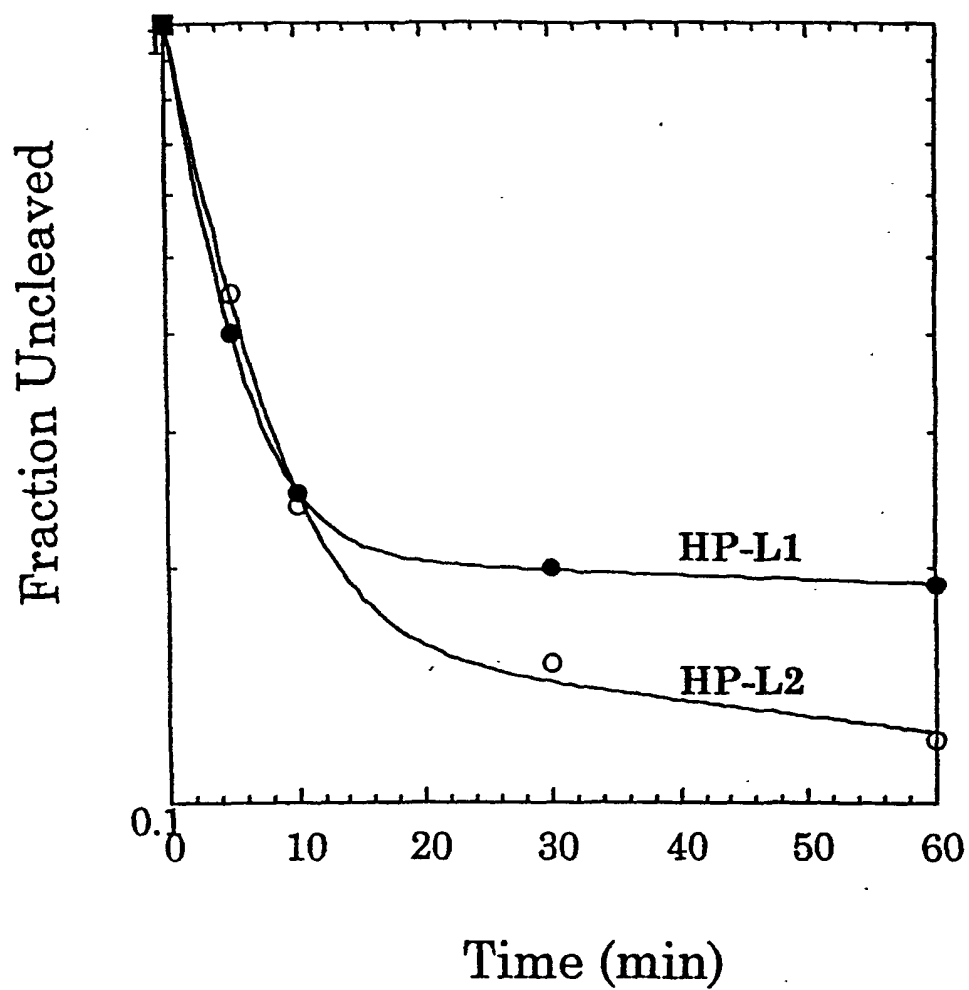


FIG. 70.

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FIG. 71b.

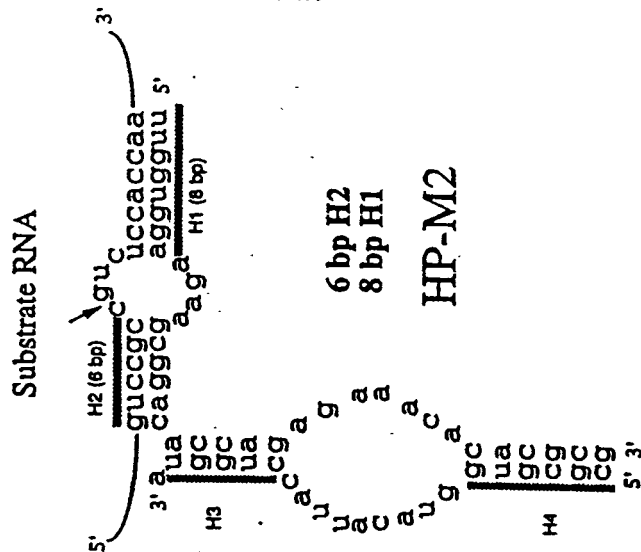
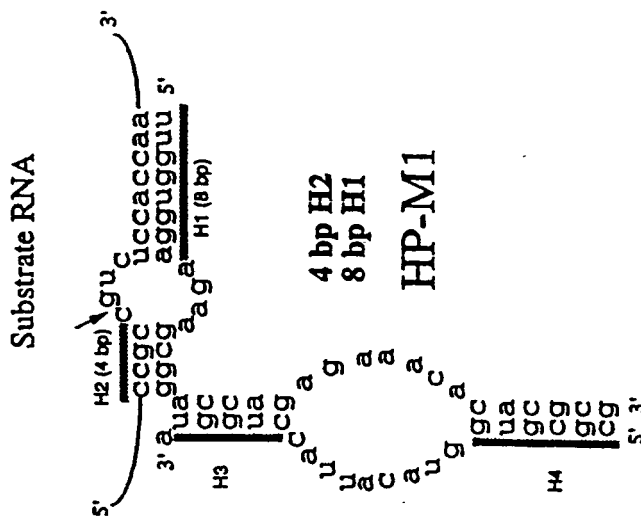


FIG. 71a.



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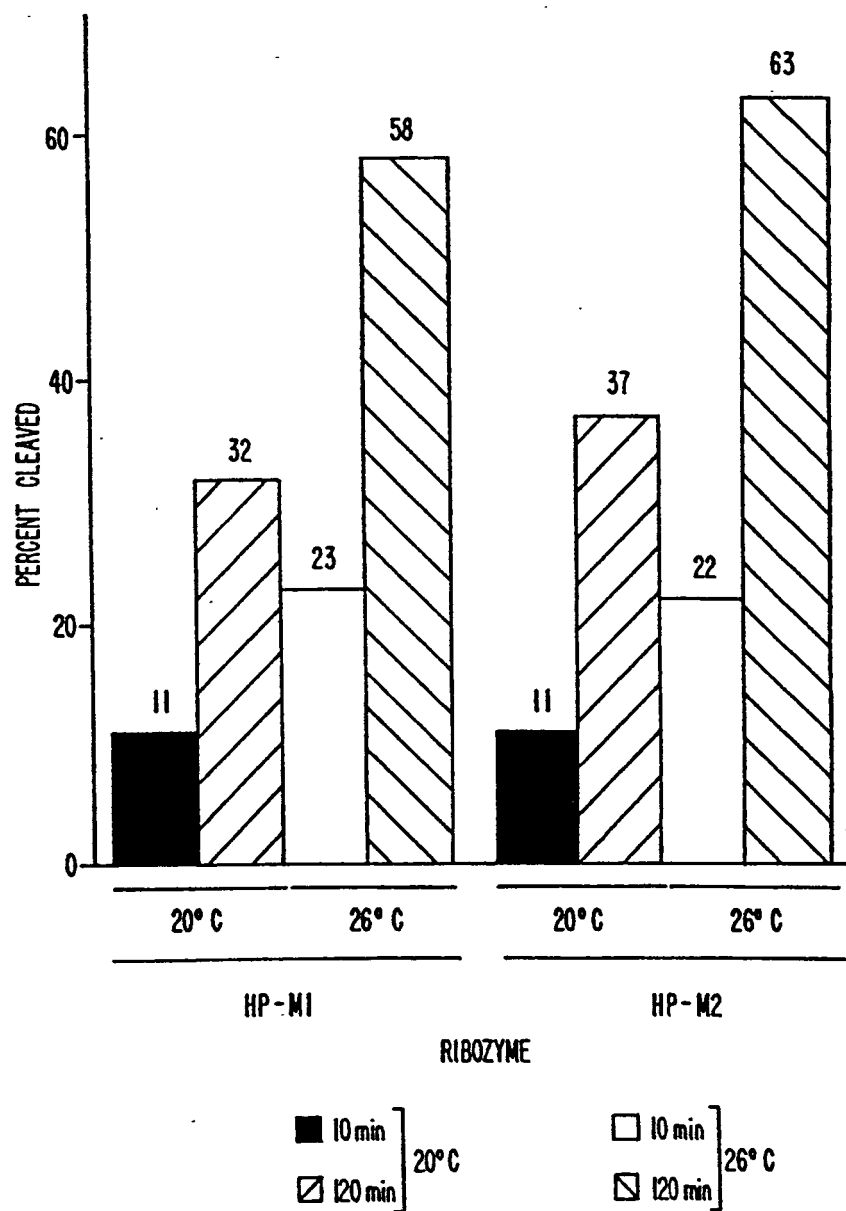
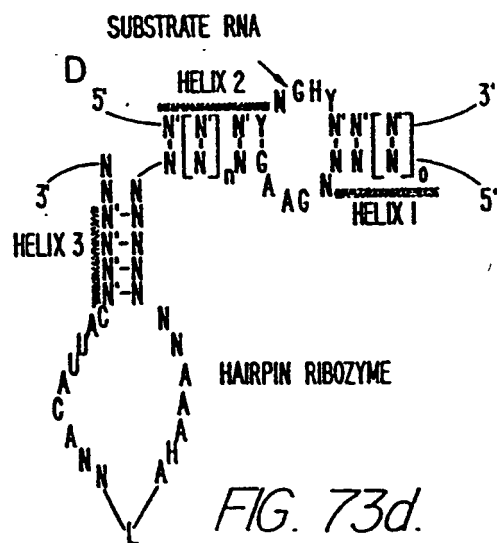
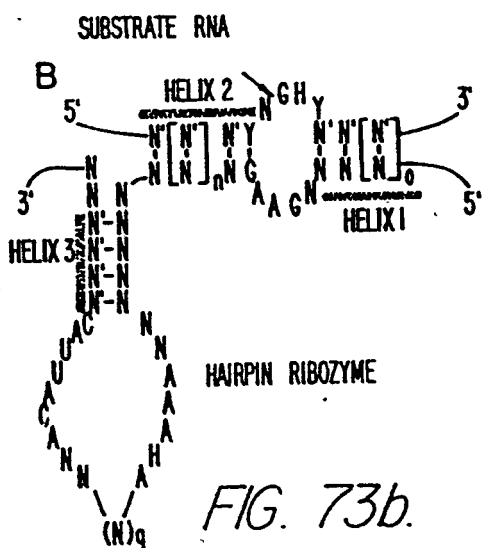
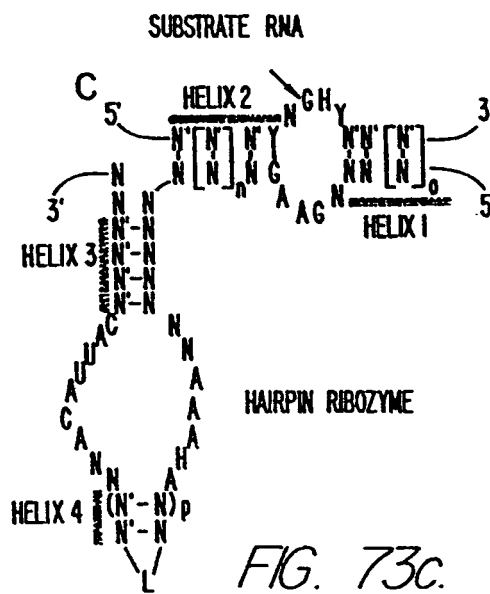
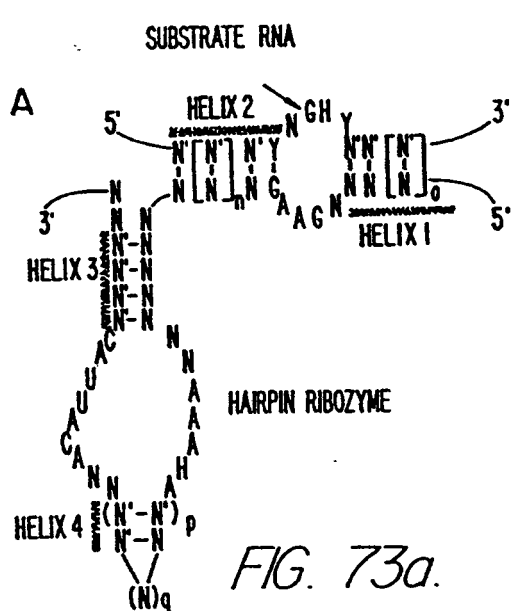


FIG. 72.

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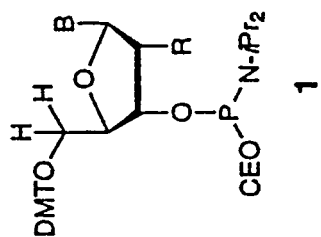


SUBSTITUTE SHEET (RULE 26)

NUC 37976

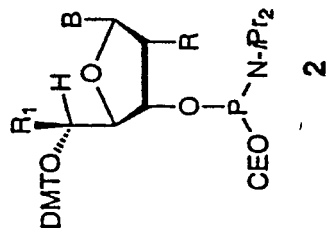
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FIG. 75a.



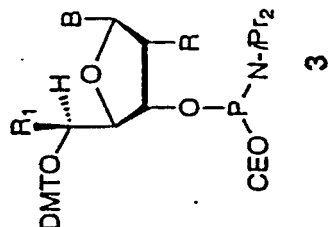
D-Ribose Family

FIG. 75b.



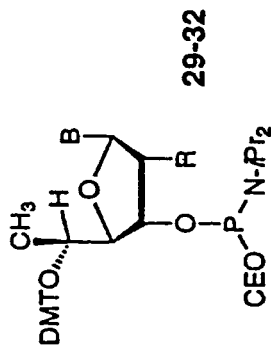
D-Allose Family

FIG. 75c.



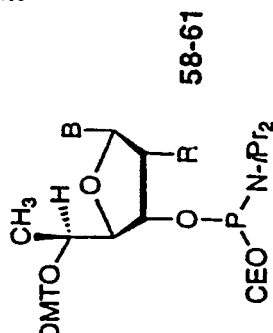
L-Talose Family

FIG. 75d.



D-Allose

FIG. 75e.



L-Talose

B = Protected A, C, G, U, T, 2AP, I, DiAP, P etc.

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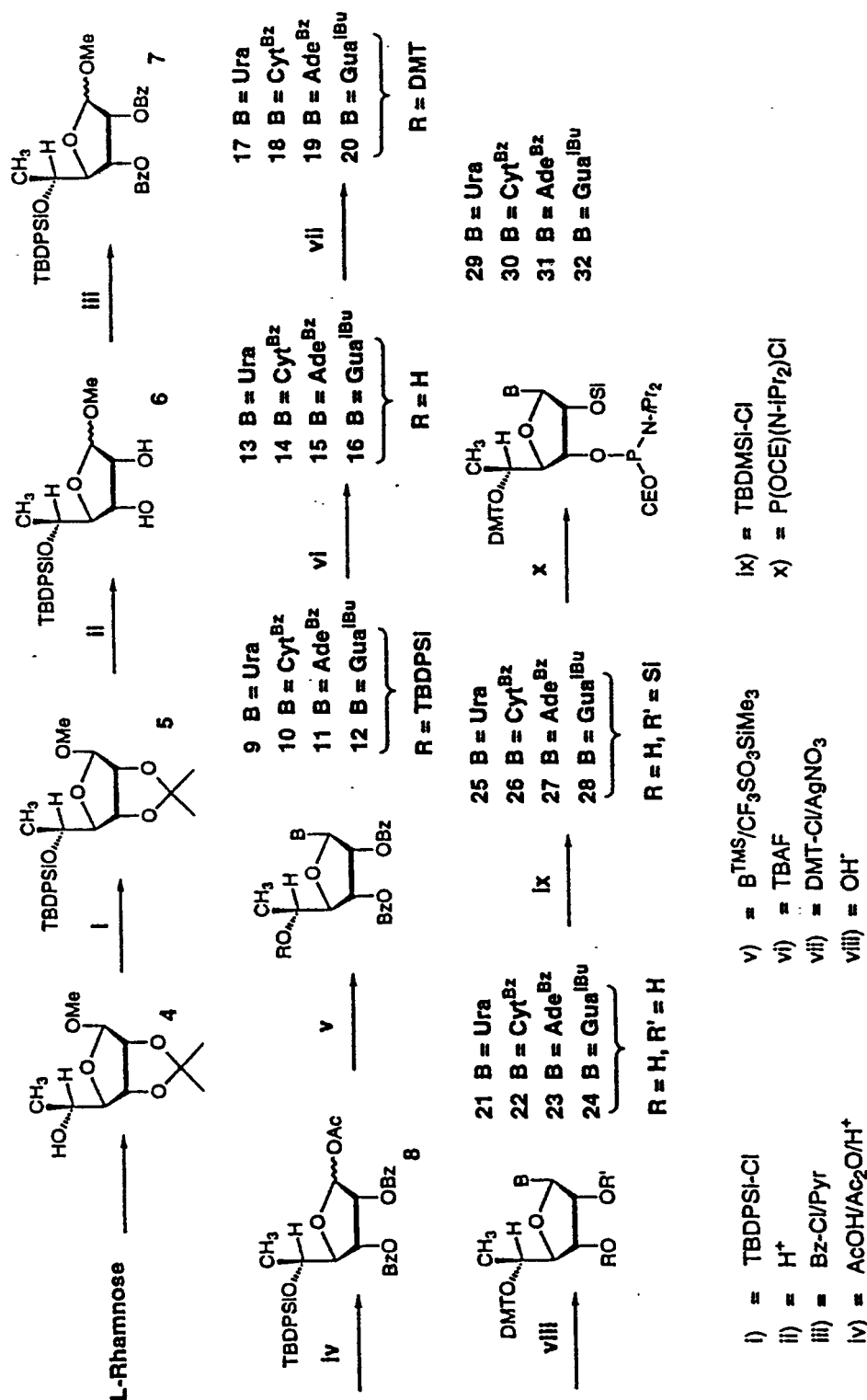


FIG. 76.

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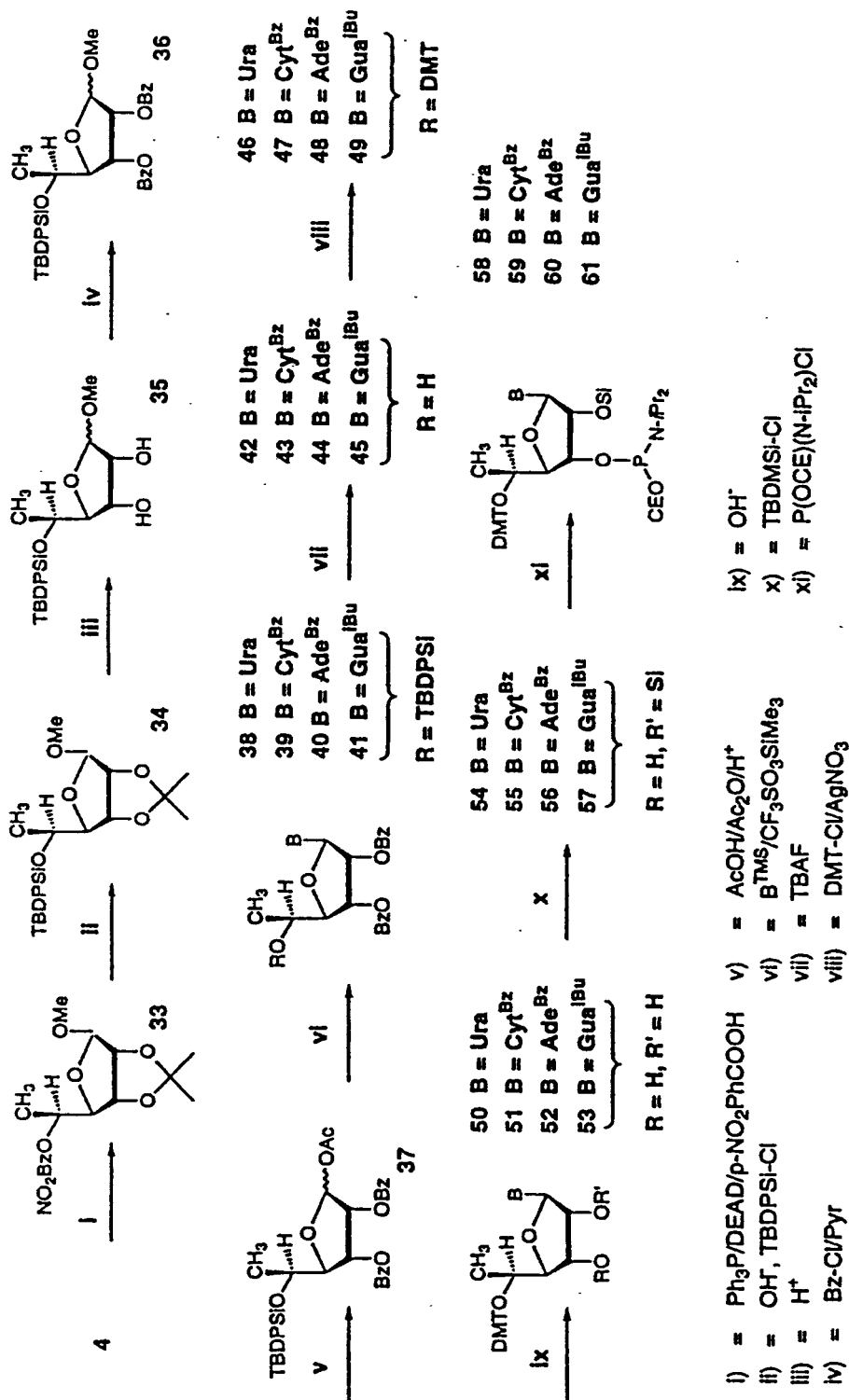
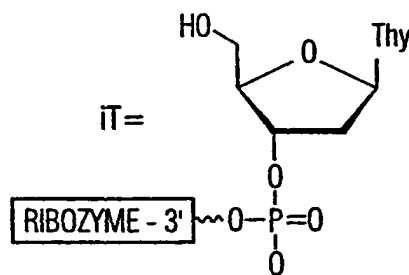
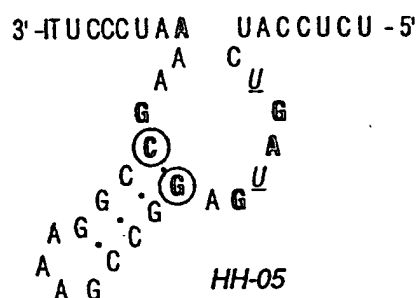
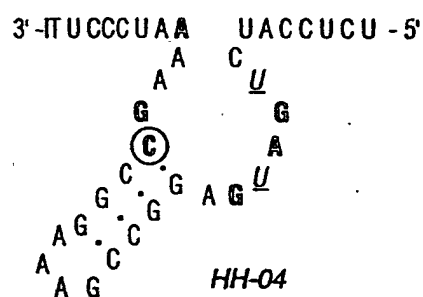
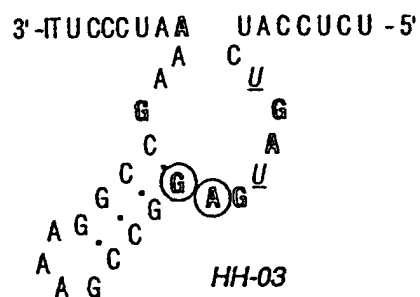
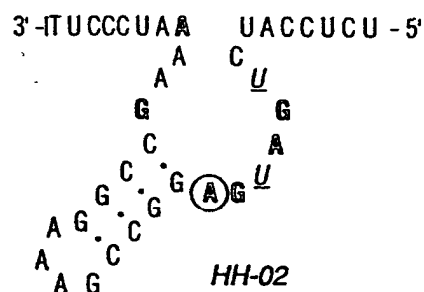
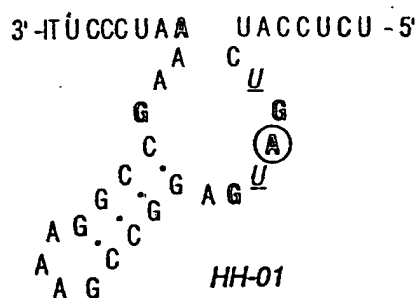


FIG. 77.

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FIG. 78.



N=2'-O-Me	N = RIBO
<u>U</u> =2'-NH ₂ U	(N) = TALO

WHERE THE ALPHABET "N" REPRESENTS A NUCLEOTIDE, A, U, G, OR C
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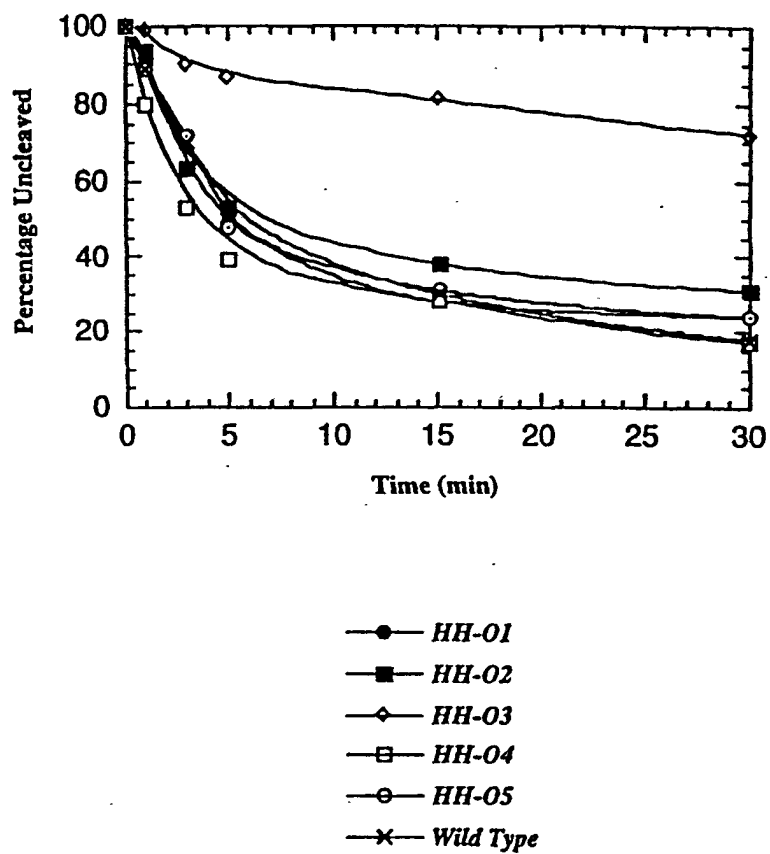


FIG. 79.

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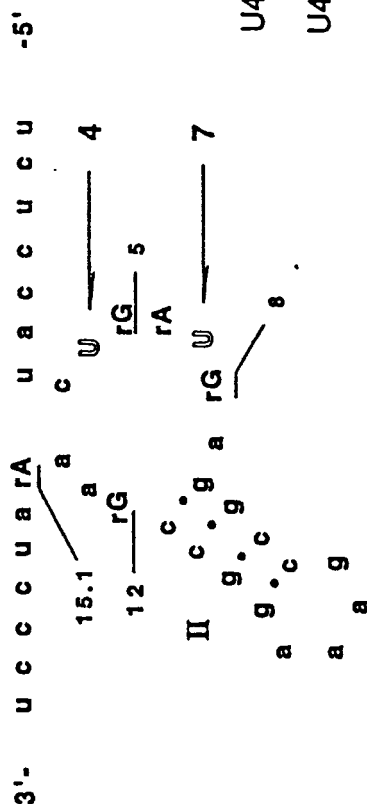


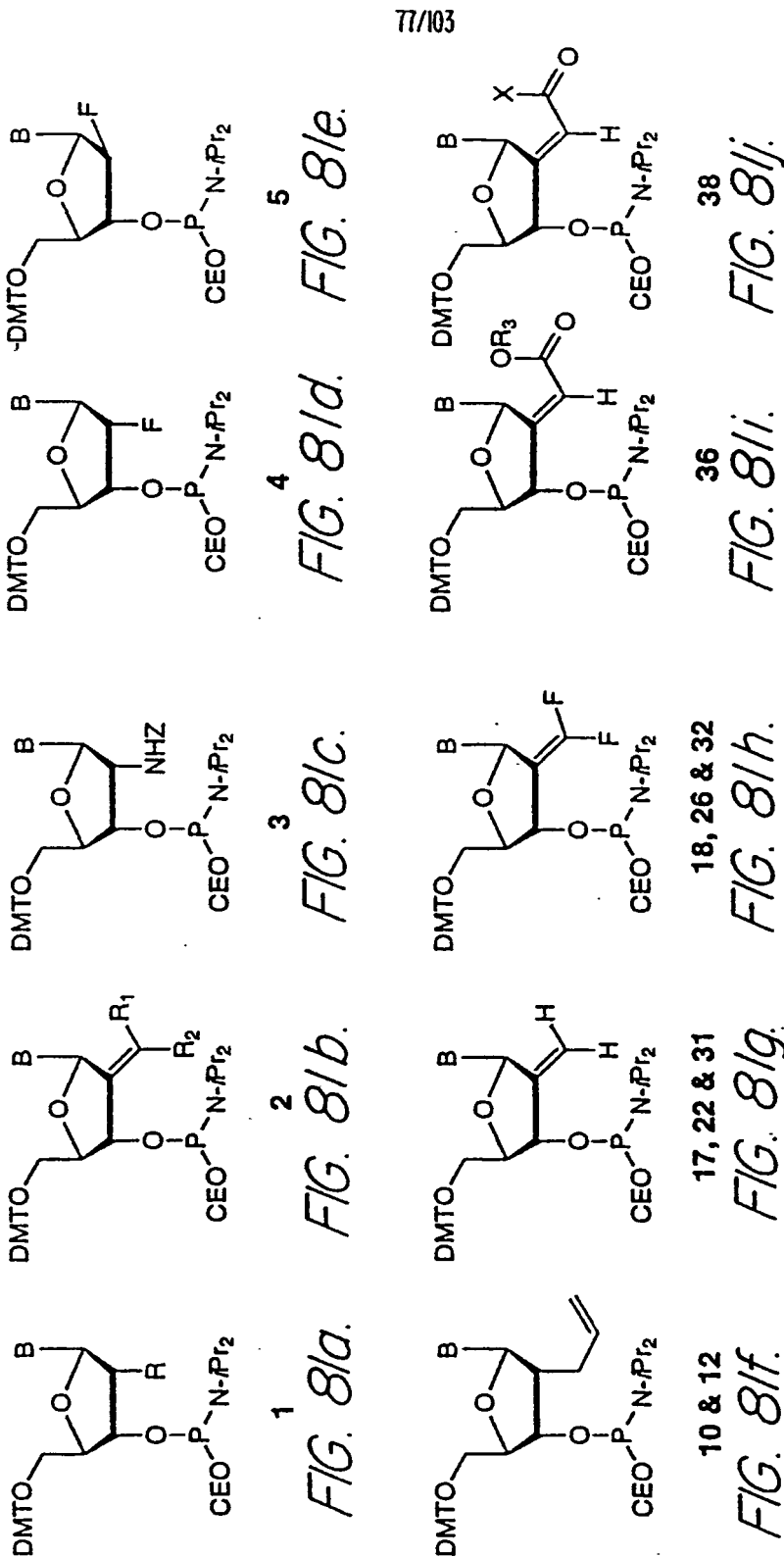
Table 1 Entries

U4 & U7 = 2'-C-Allyl-U	12-14
U4 & U7 = 2'-F-ribo-U	9-11
U4 & U7 = 2'=CH ₂ -U	3-5
U4 & U7 = 2'=CF ₂ -U	6-8
U4 & U7 = 2'-dU	21-22
U4 & U7 = 2'-F-ara-U	15-17
U4 & U7 = 2'-NH ₂ -U	18-20
U4 & U7 = 2'-O-Me-ribo-U	2

Lower case = 2'-O-Me
rN = ribonucleotide

FIG. 80.

SUBSTITUTE SHEET (RULE 26)



SUBSTITUTE SHEET (RULE 26)

B = Protected A, C, G, U, T, 2AP, I, DIAP, P etc.

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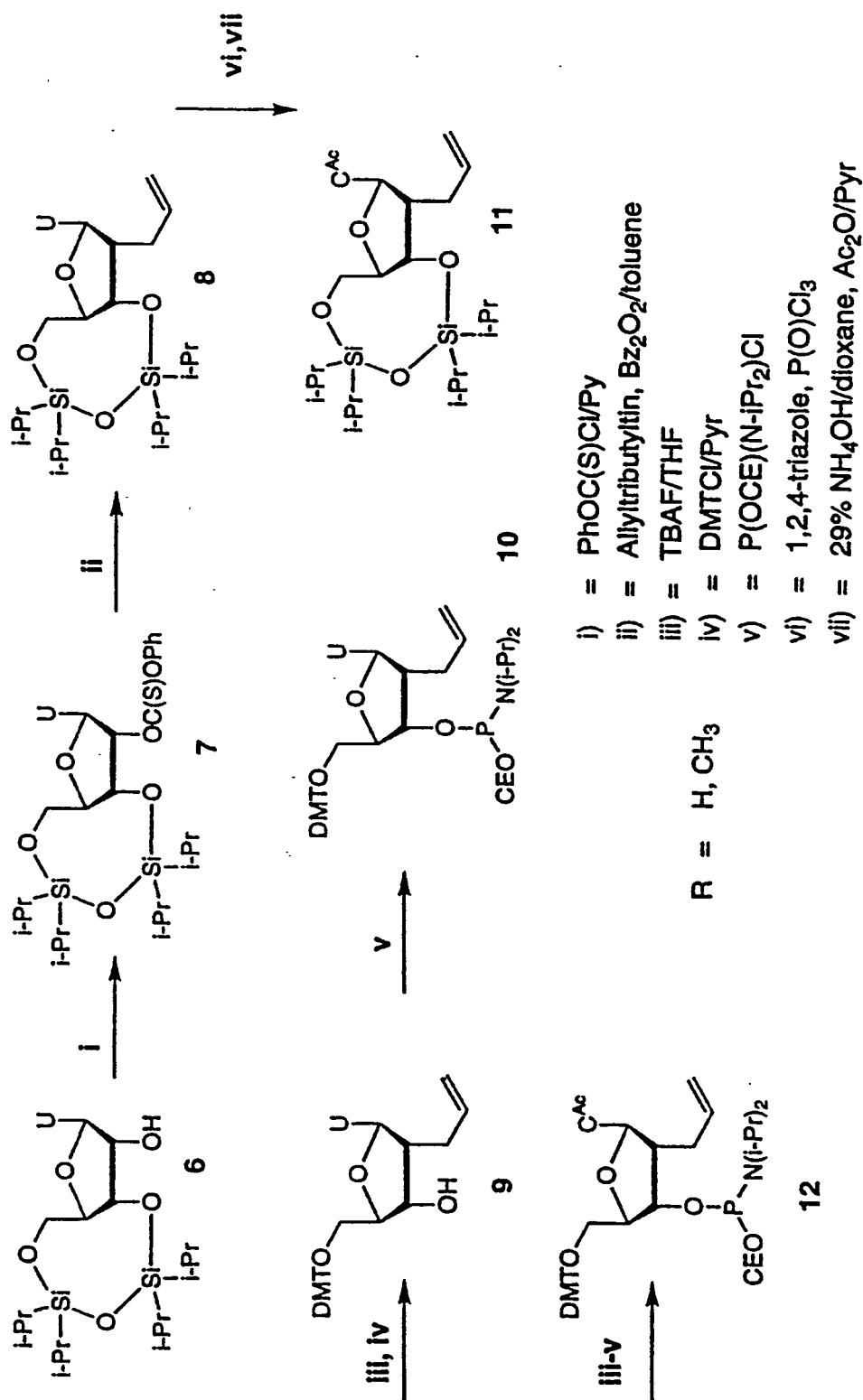
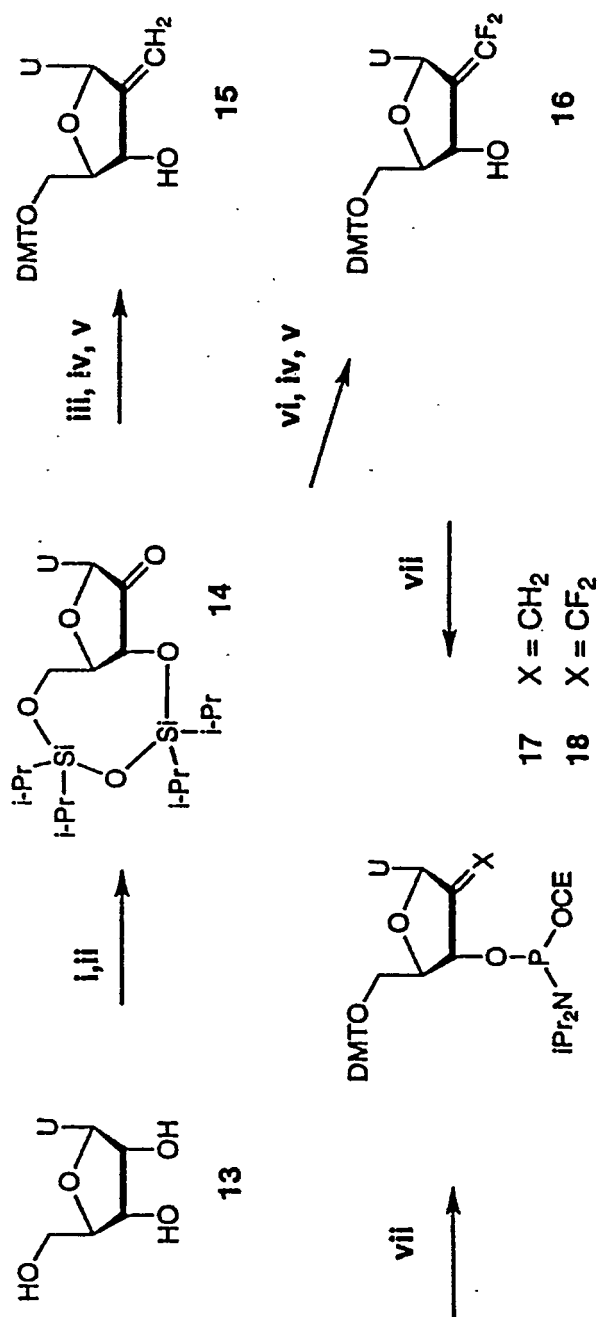


FIG. 82.

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FIG. 83.



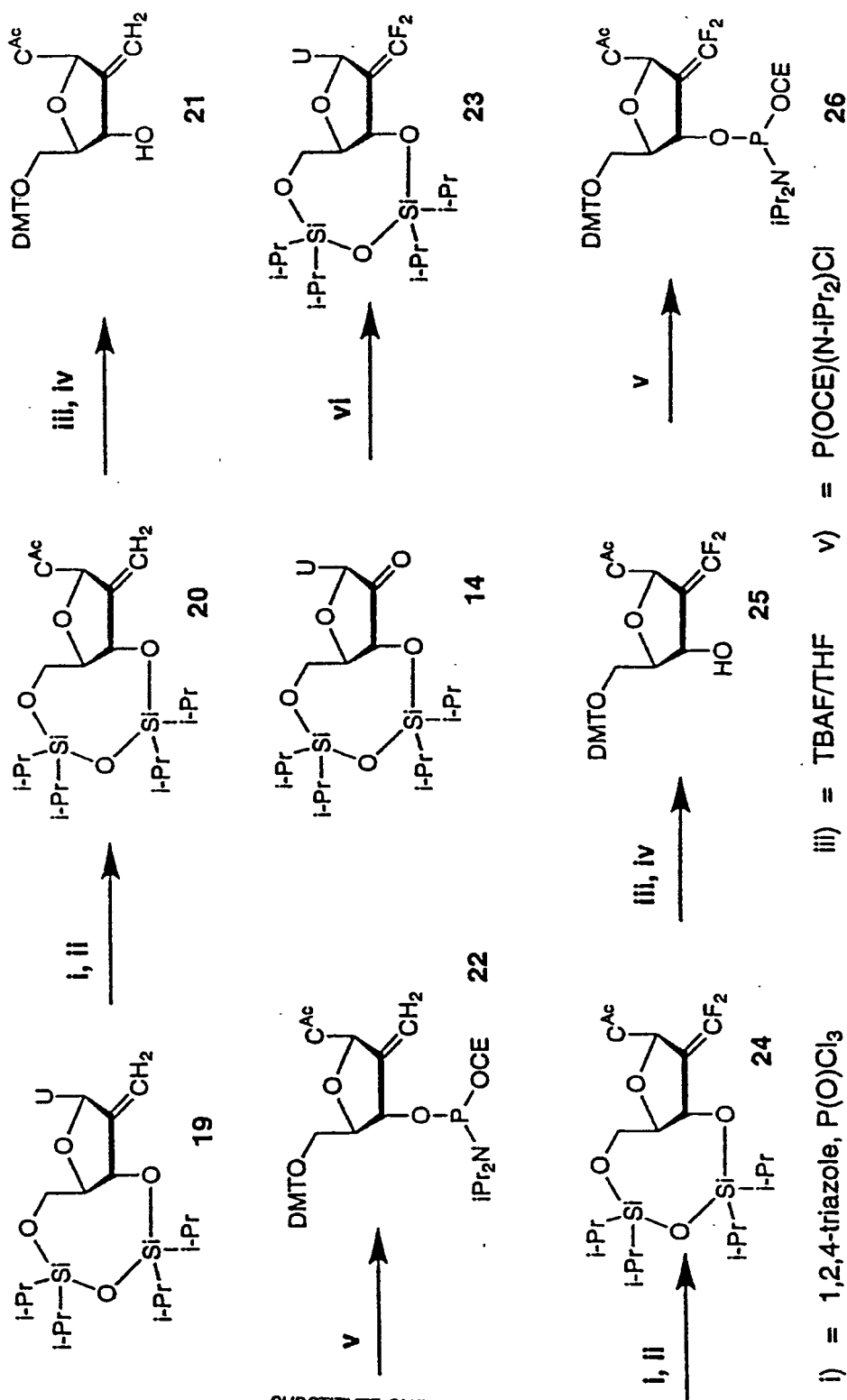
- i) = Markiewicz reagent
 ii) = DMSO & Ac₂O
 iii) = Ph₃PCH₃I
 iv) = TBAF/THF
 v) = DMTCl/Pyr
 vi) = Ph₃P, ClCF₂COONa
 vii) = P(OCE)(N-iPr₂)Cl

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FIG. 84.



i) = 1,2,4-triazole, P(O)Cl₃
 ii) = 29% NH₄OH/dioxane, Ac₂O/Pyr
 iii) = TBAF/THF
 iv) = DMTCl/Pyr
 v) = P(OCE)(N-IPr₂)Cl
 vi) = Ph₃P, ClCF₂COONa

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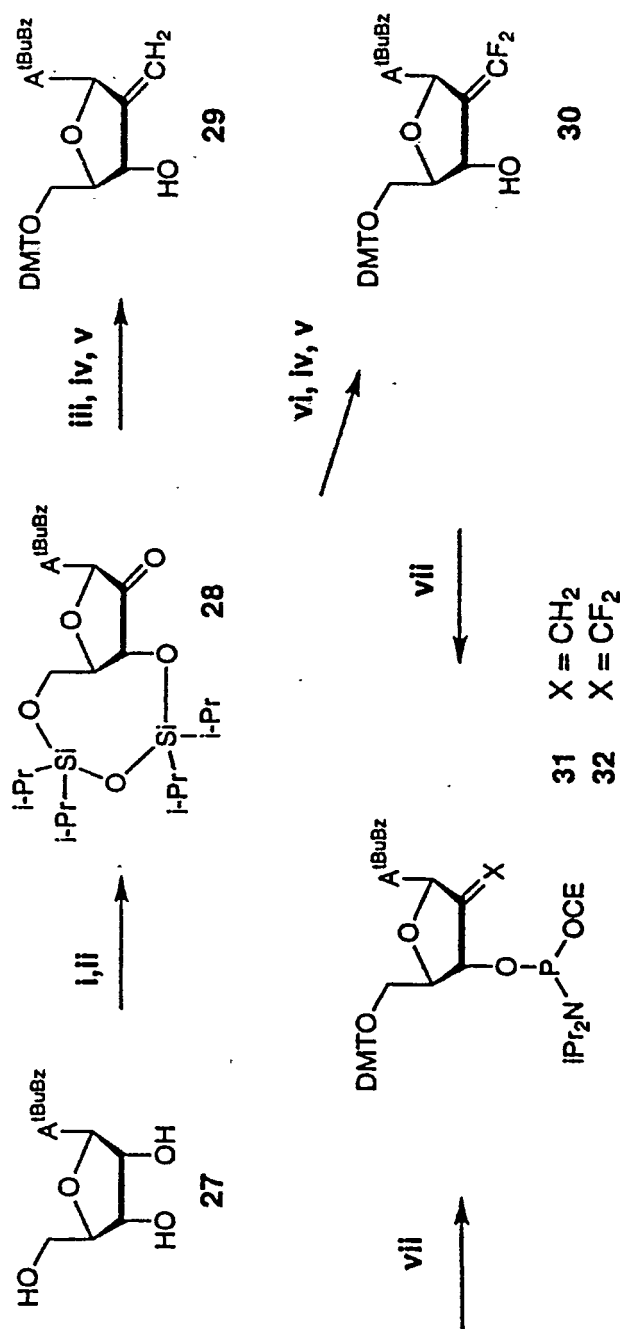
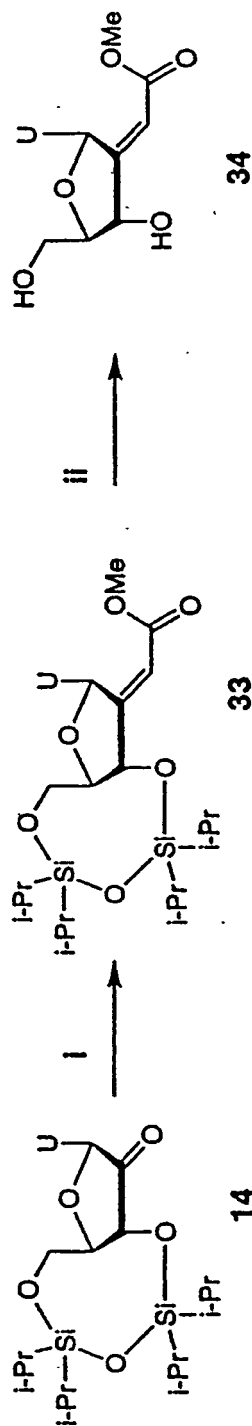


FIG. 85.

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- i) = $\text{Ph}_3\text{PC}=\text{CHC}(\text{O})\text{OCH}_3\cdot\text{OAc}$
 ii) = $\text{NEt}_3\cdot 3 \text{ HF}$
 iii) = DMTCI/Pyr
 iv) = $\text{P}(\text{OCE})(\text{N-IPr}_2)\text{Cl}$
 v) = MeOH/NaOH

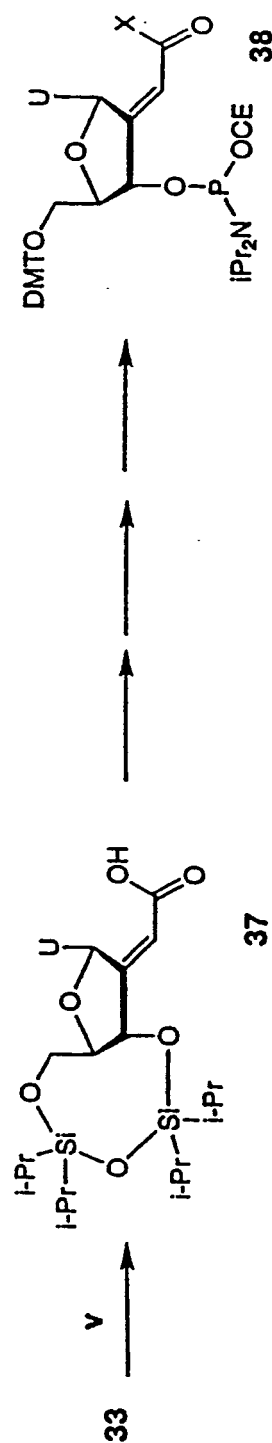
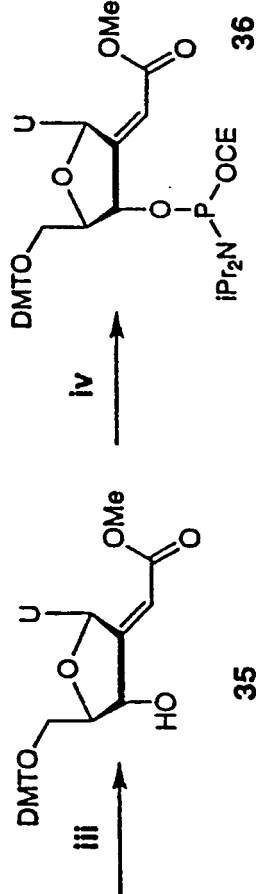


FIG. 86.

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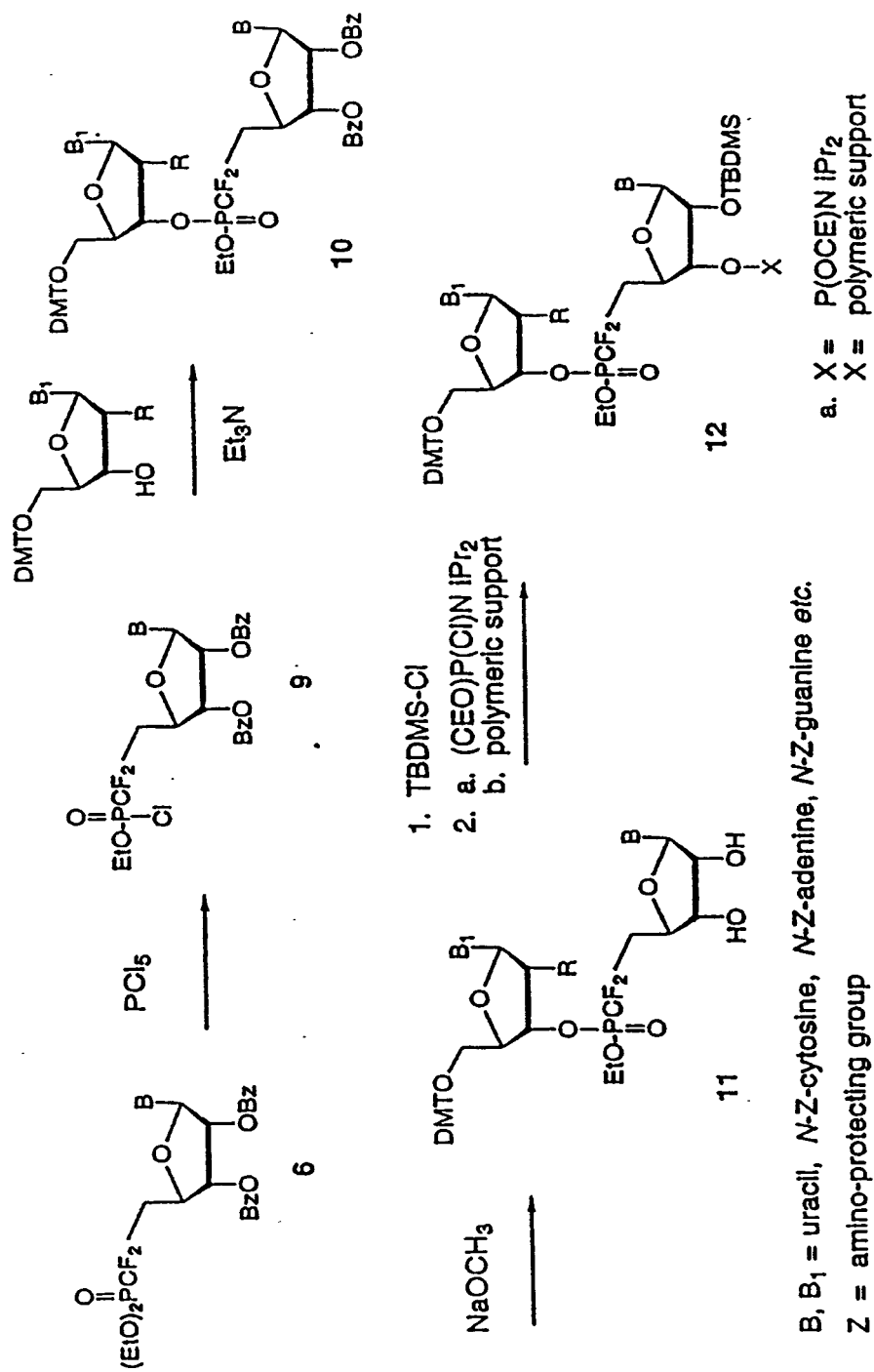
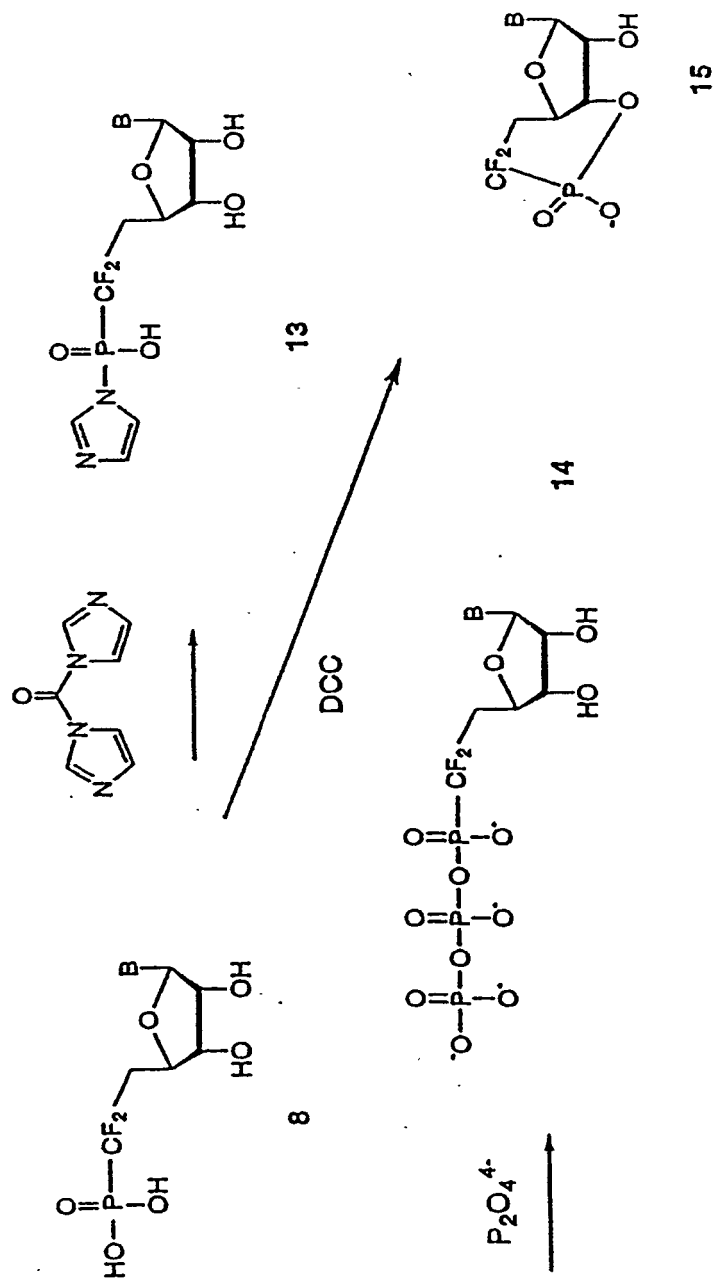


FIG. 88.

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B = uracil, N-Z-cytosine, N-Z-adenine, N-Z-guanine etc.

Z = amino-protecting group

FIG. 89.

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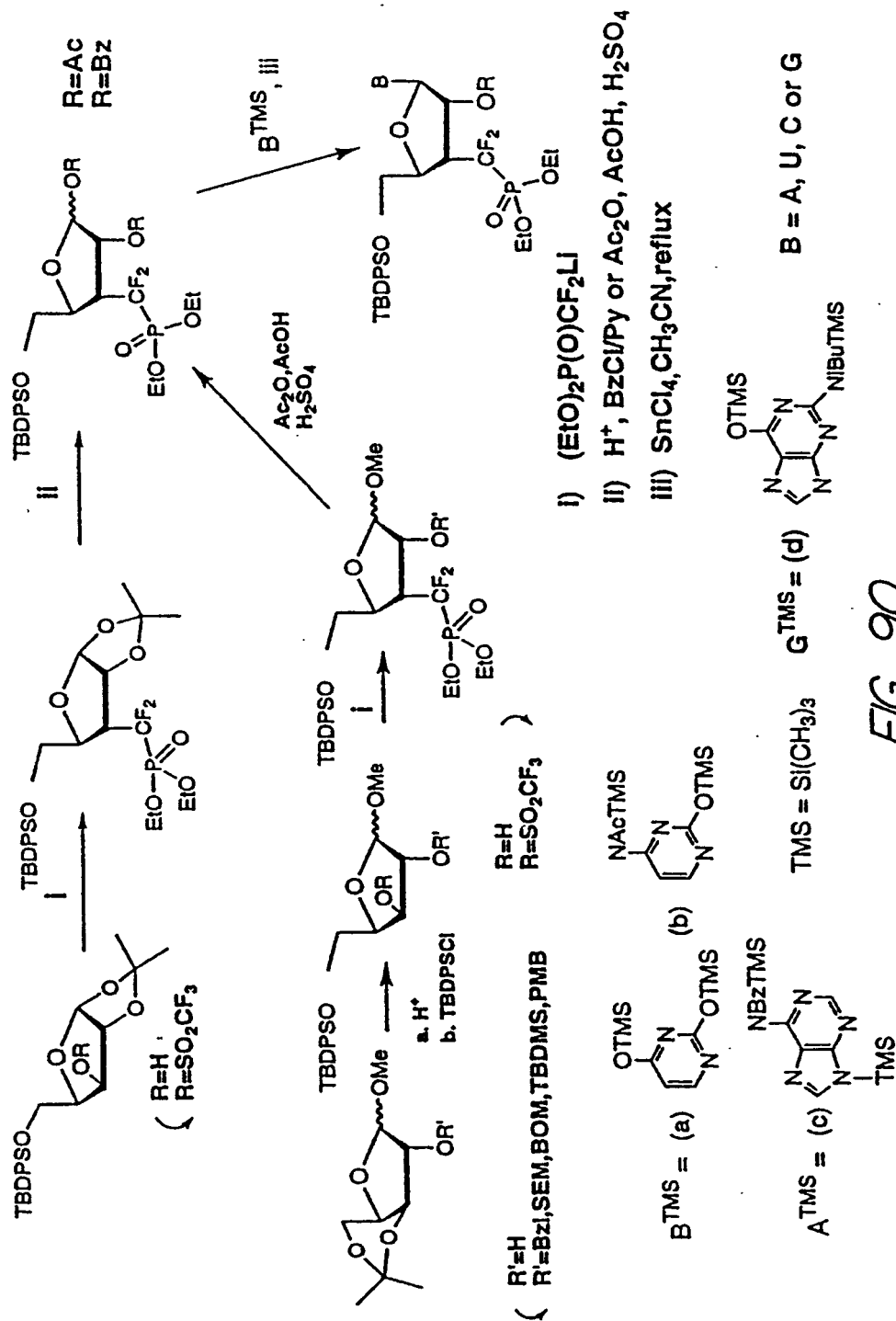


FIG. 90.

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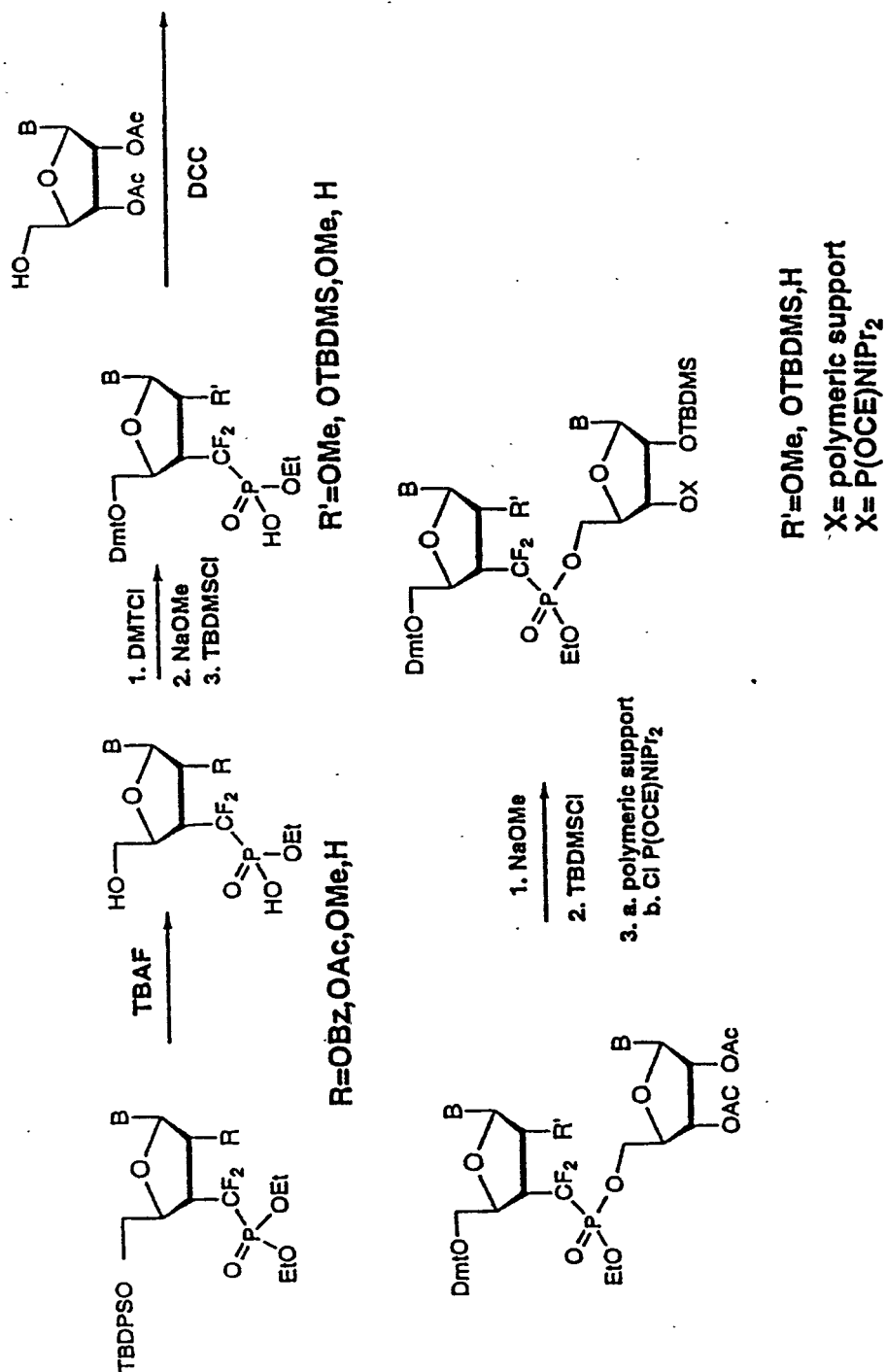
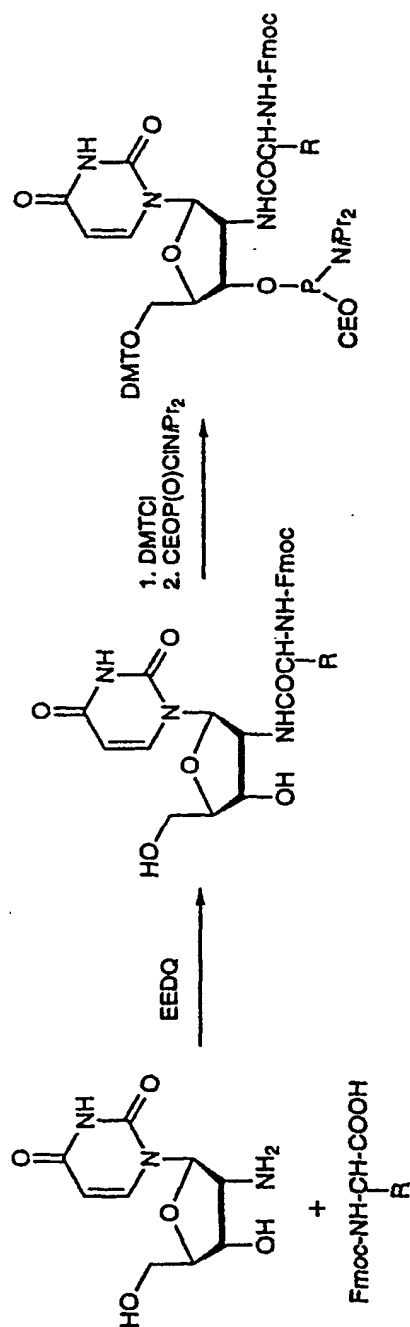


FIG. 91.

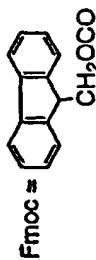
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SUBSTITUTE SHEET (RULE 26)

EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline



R = CH₃, CH₂- (phe), (CH₂)₄NH-Fmoc, (CH₂)₄NH-CBZ, CH₂COOBzl
 (ala) (lys) (asp)

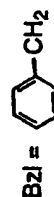
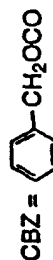
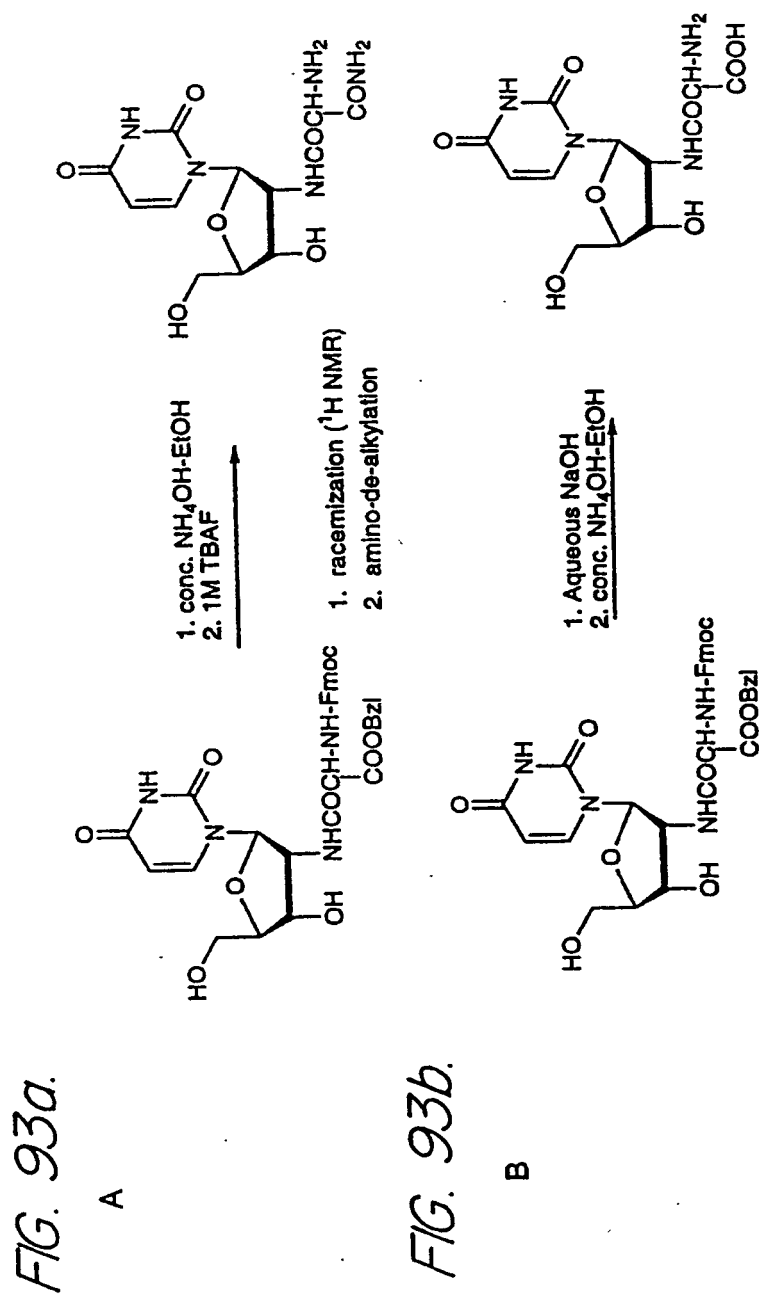


FIG. 92.

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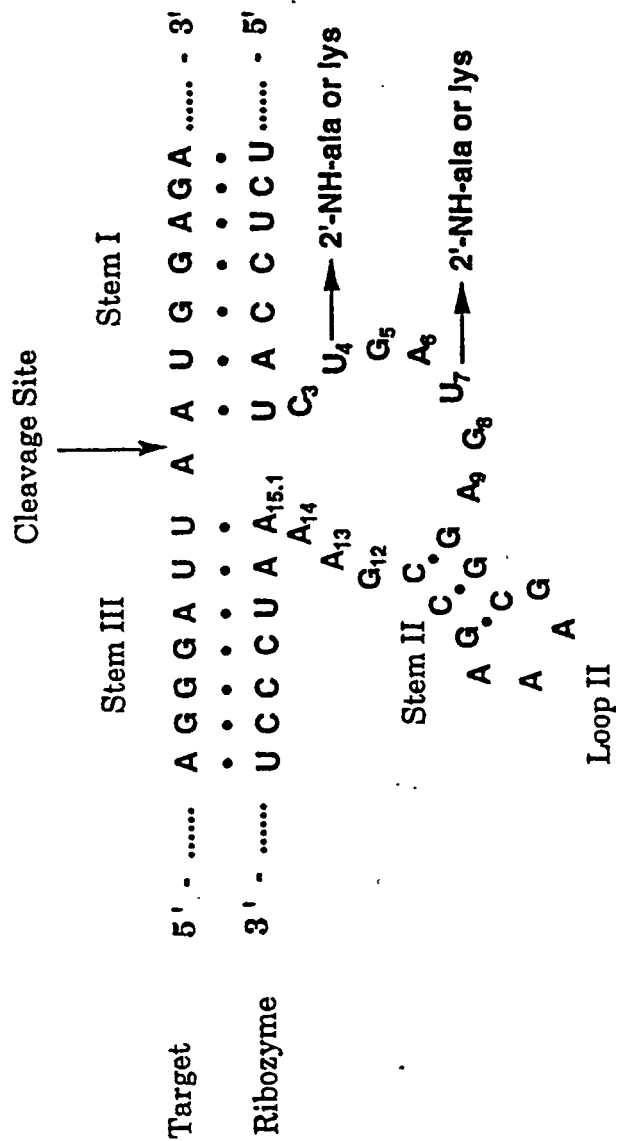
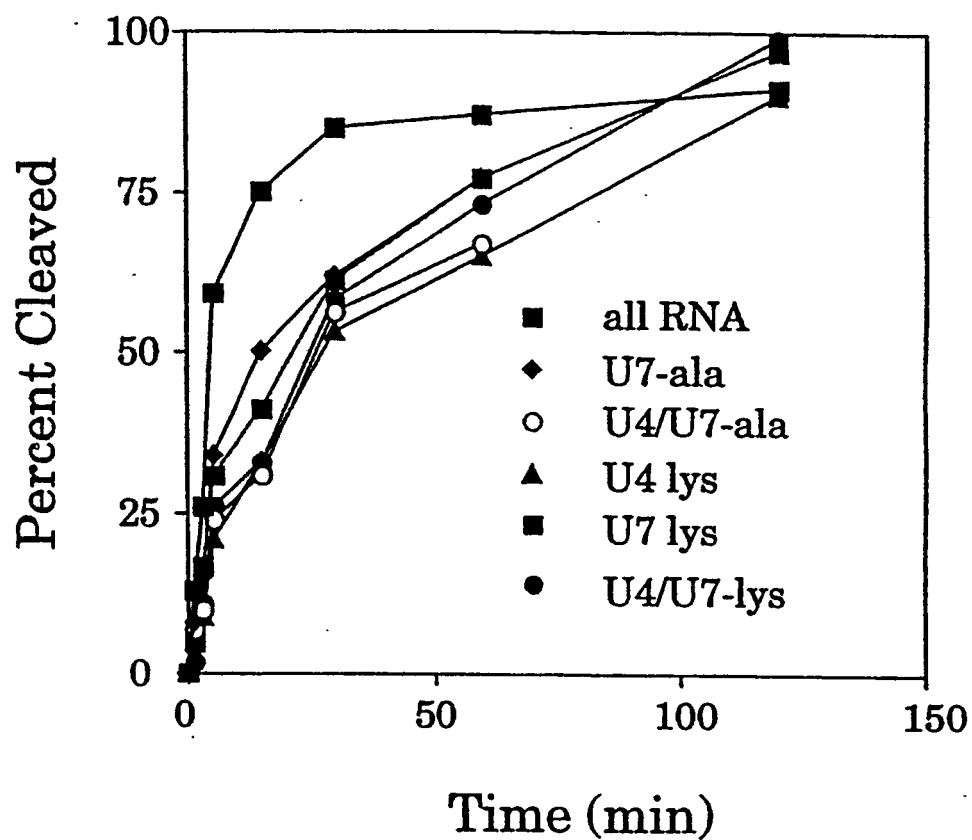


FIG. 94.

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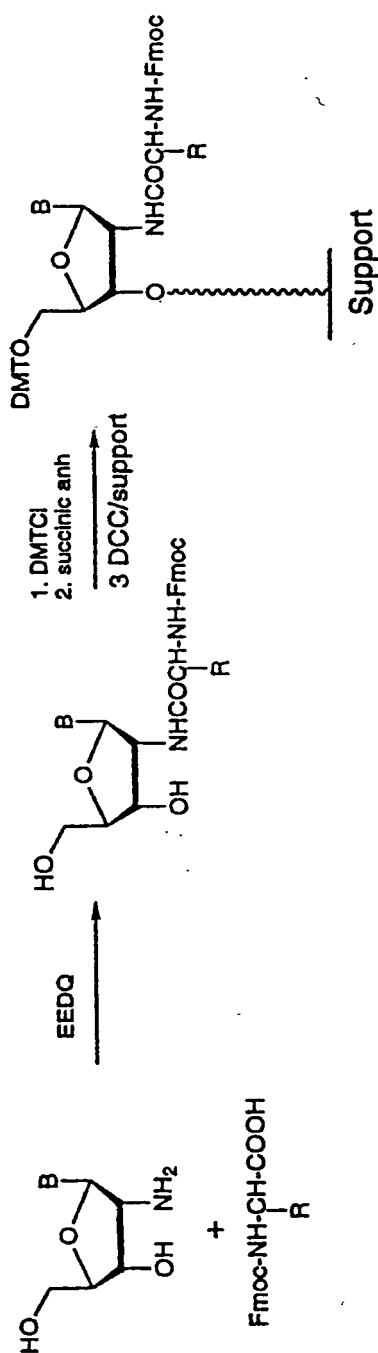
[Ribozyme] = 40 nM [Substrate] = ~1nM

FIG. 95.

SUBSTITUTE SHEET (RULE 26)

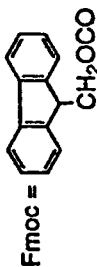
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B = Ura, Cyt^{bz}, Ade^{bz}, Gua^{ibu}, mod. base, H

EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline



R = CH₃, CH₂-C₆H₅ (phe), (CH₂)₄NH-CBZ, CH₂COOBzl (asp)

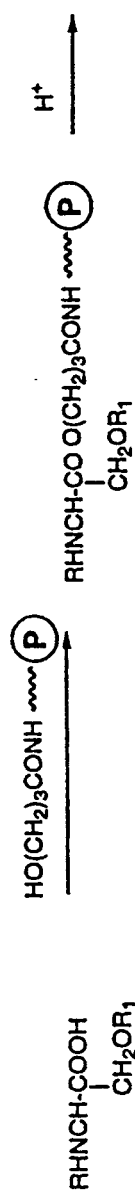
CBZ = C₆H₅-CH₂OCO

Bzl = C₆H₅-CH₂

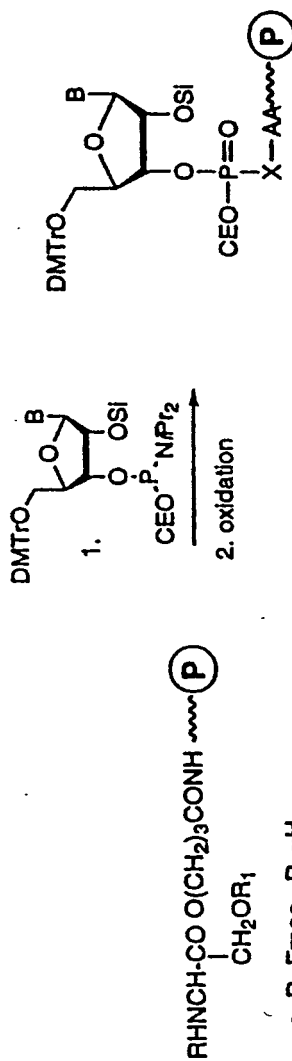
FIG. 96.

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a R=Fmoc, R₁=DMTr
 b R=MMTr, R₁=Bz



a R=Fmoc, R₁=H
 b R=H, R₁=Bz

a X=O, AA=CH₂CH(NHFmoc)CO
 b X=NH, AA=CH(CH₂OBz)CO

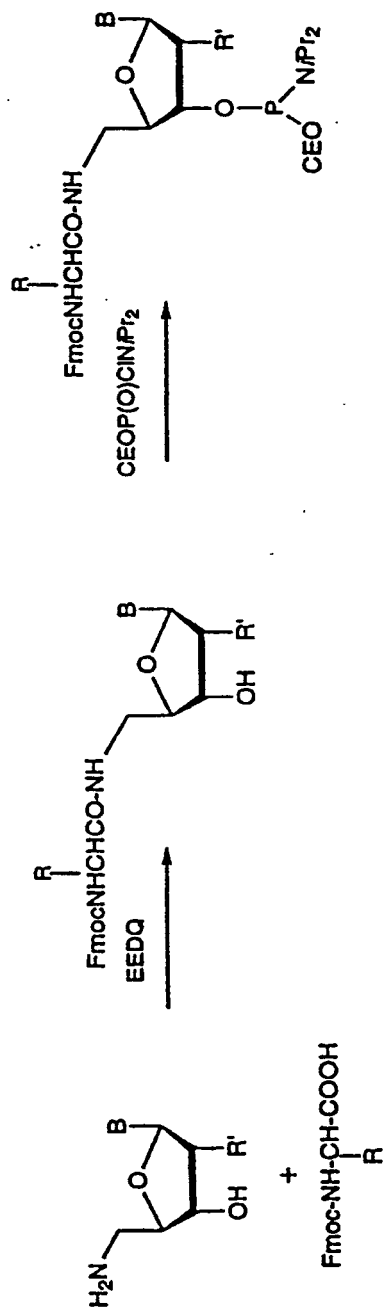
B= Ura, Cyt^{bz}, Ade^{bz}, Gua^{ibu}, mod. base, H

FIG. 97.

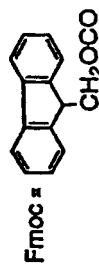
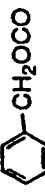
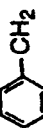
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EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline

R = CH₃, CH₂-, (CH₂)₄NH-Fmoc, (CH₂)₄NH-CBZ, CH₂COOBzl (ala), (lys), (asp)CBZ = Bzl = 

R' = H, OMe, OTBDMSi

B = Ura, Cyt^{bz}, Ade^{bz}, Gua^{ibu}, mod. base, H

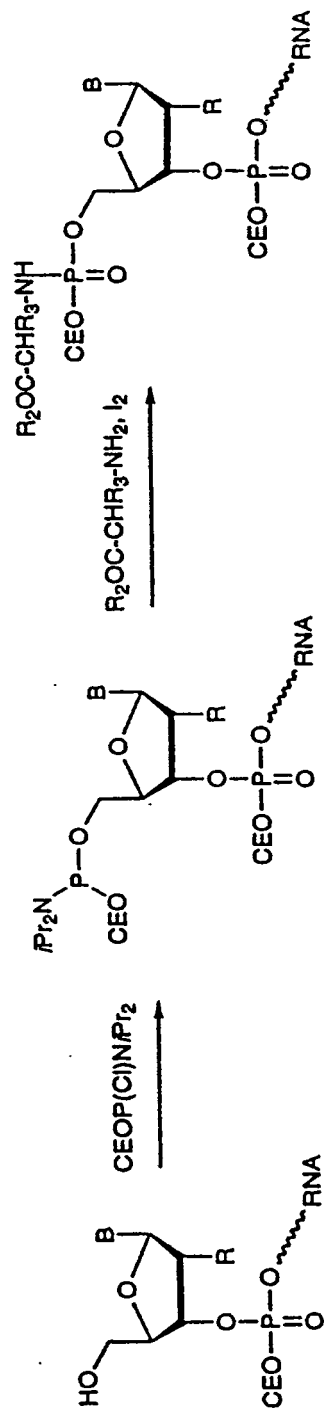
FIG. 98.

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FIG. 99.



B = Ura, Cyt^{bz}, Ade^{bz}, Gua^{ibu}, mod. base, H
 R = H, OCH₃, OTBDMS, Hal, NHR₁
 R₂ = OBzl, peptidyl

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FIG. 100.

Reversion of mutant RNA

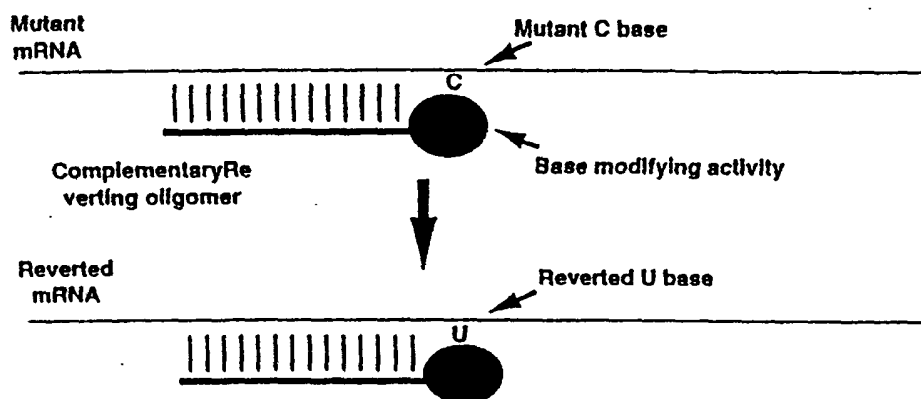
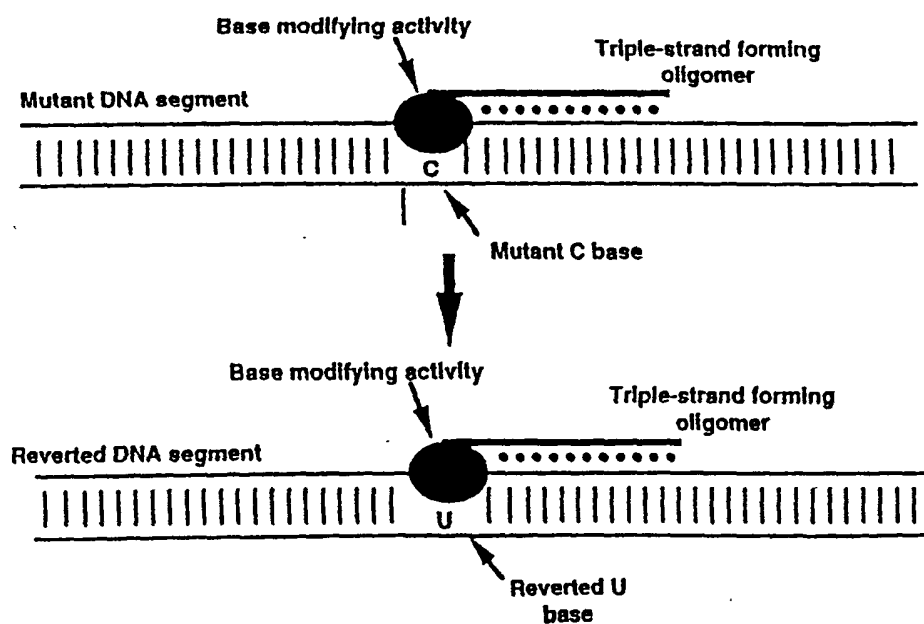


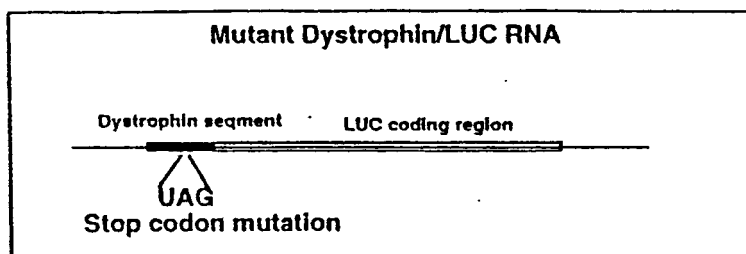
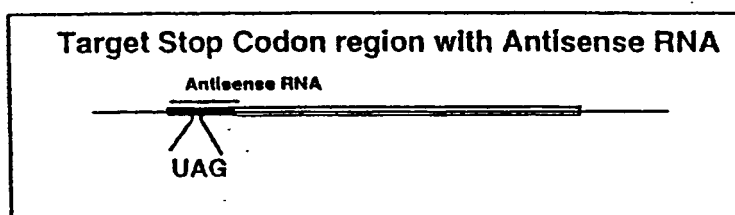
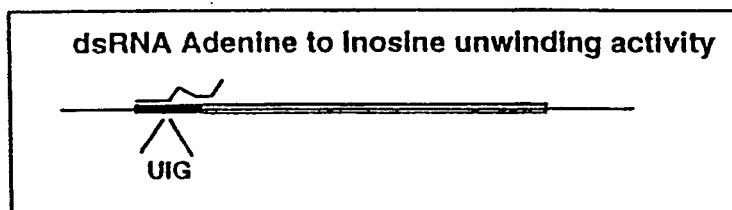
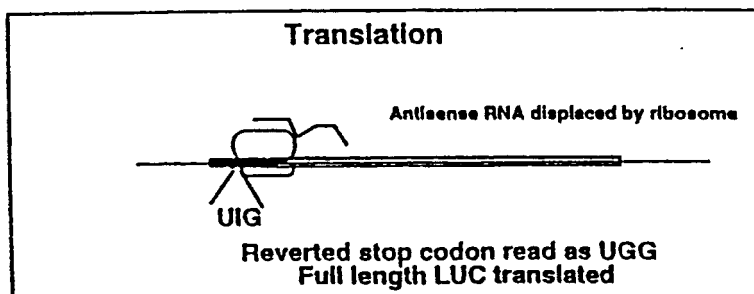
FIG. 101.

Reversion of mutant DNA



SUBSTITUTE SHEET (RULE 26)

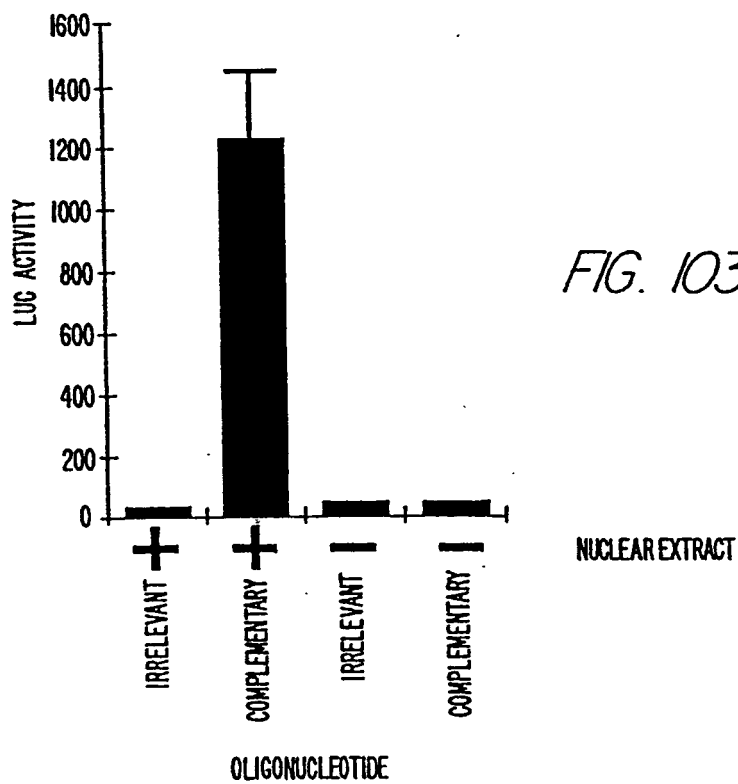
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*FIG. 102a.**FIG. 102b.**FIG. 102c.**FIG. 102d.*

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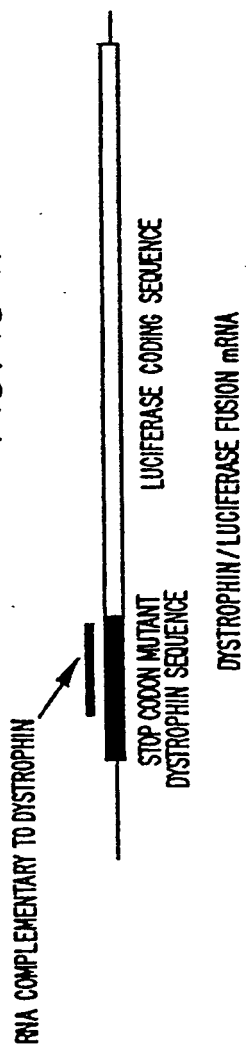


SUBSTITUTE SHEET (RULE 26)

NUC 38005

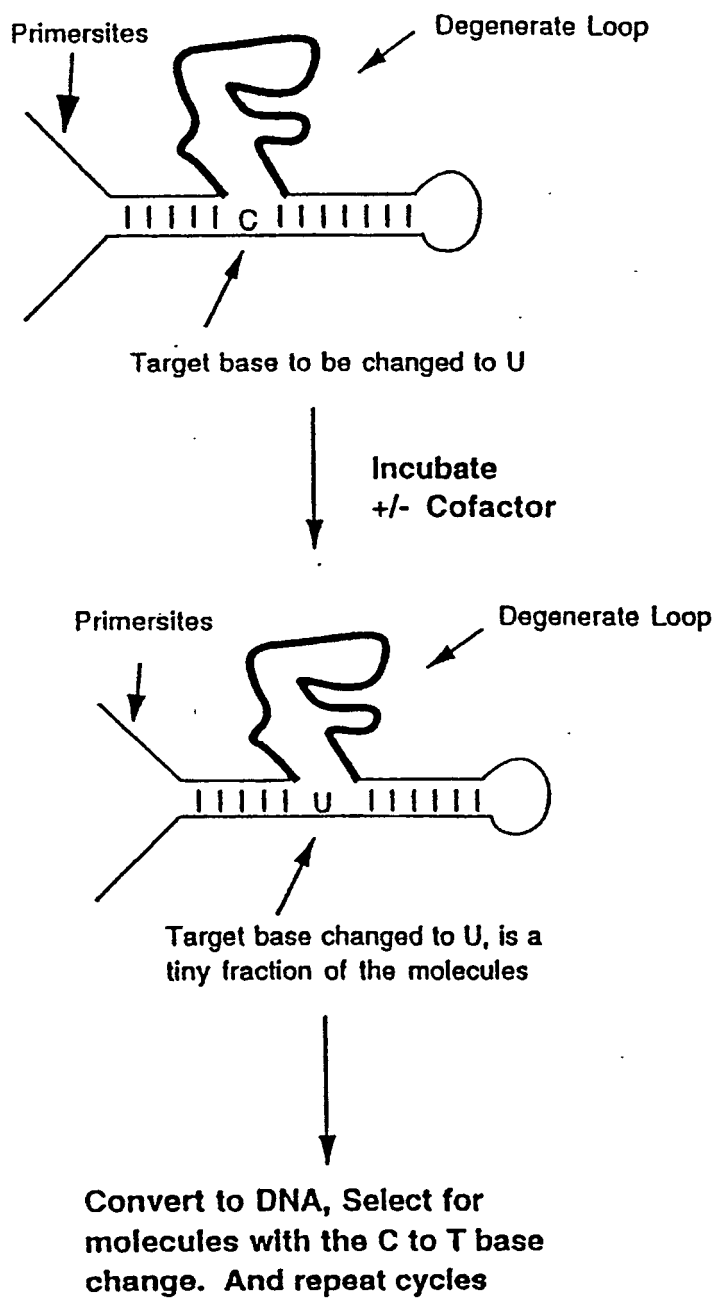
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FIG. 104.



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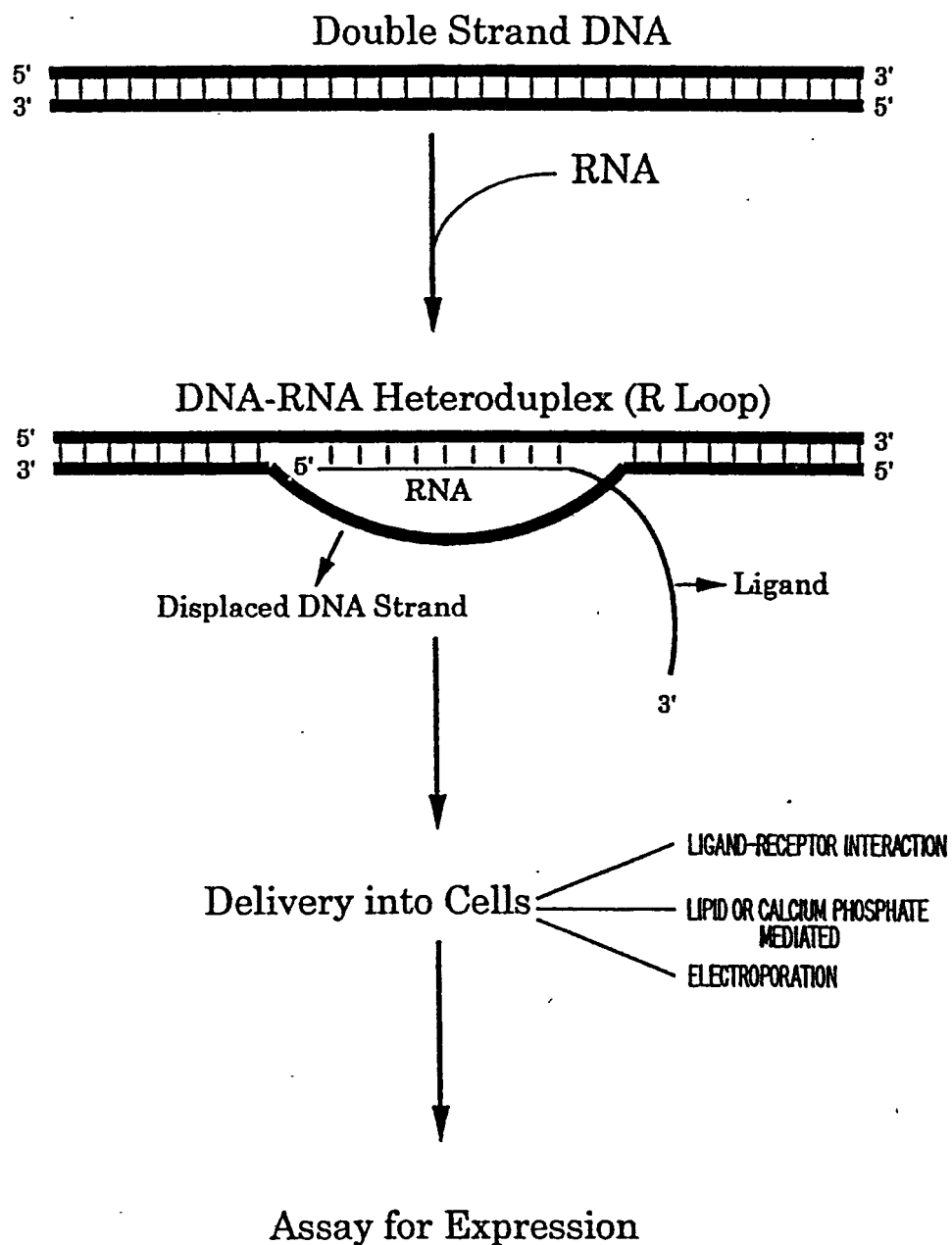
FIG. 105.



SUBSTITUTE SHEET (RULE 26)

NUC 38007

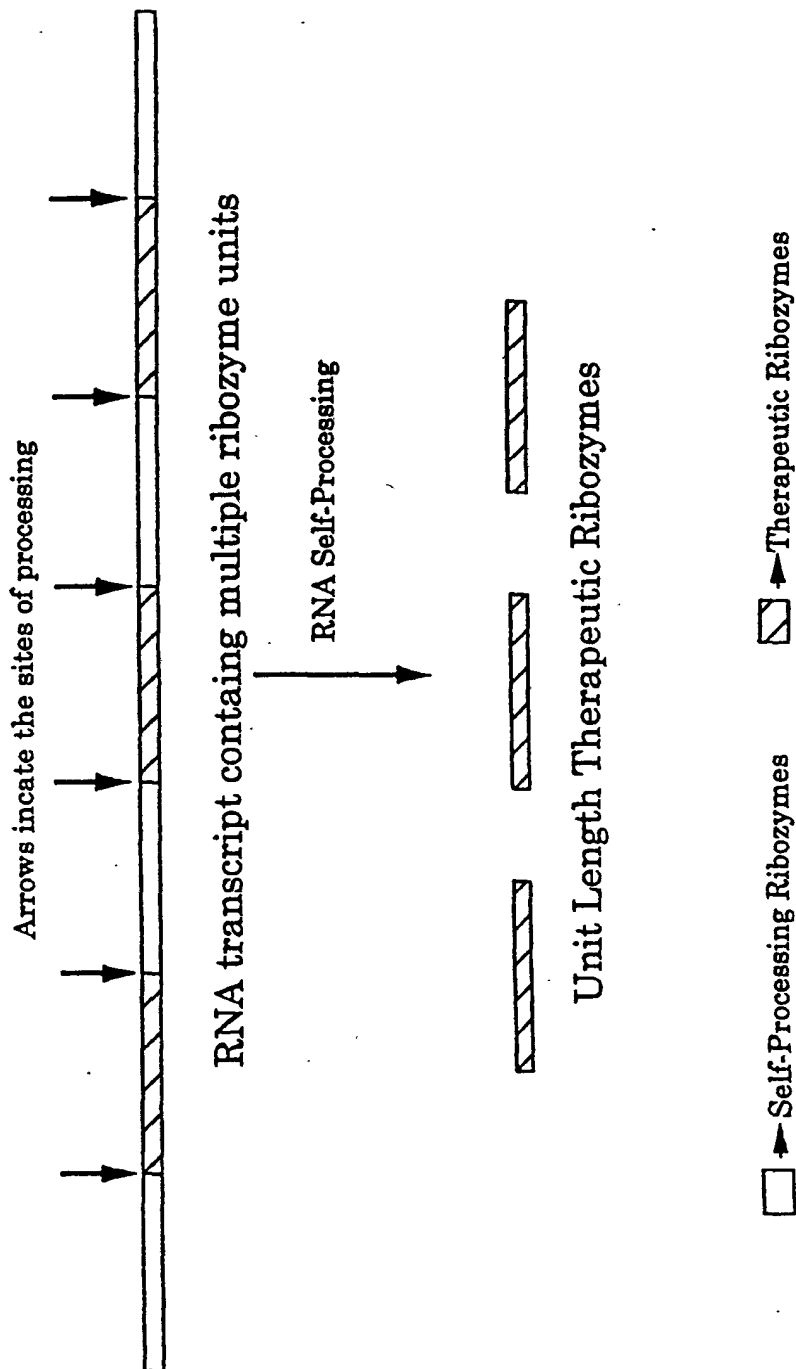
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*FIG. 106.*

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FIG. 107.



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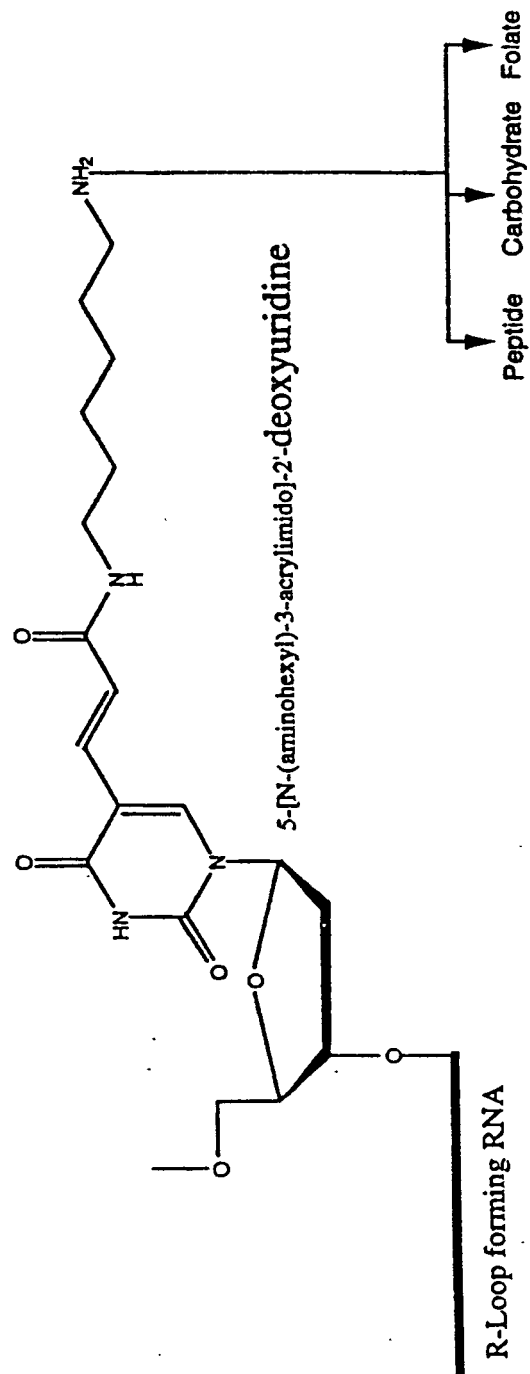


FIG. 108.

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